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Thioredoxins and related proteins in photosynthetic organisms: molecular basis for thiol dependent regulation

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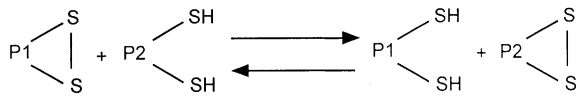
Abstract

Thioredoxins are small molecular weight disulfide oxidoreductases specialized in the reduction of disulfide bonds on other proteins. Generally, the enzymes which are selectively and reversibly reduced by these proteins oscillate between an oxidized and inactive conformation and a reduced and active conformation. Thioredoxin constitutes the archetype of a family of protein disulfide oxidoreductases which comprises glutaredoxin and protein disulfide isomerase. Thioredoxin and glutaredoxin serve many roles in the cell, including the redox regulation of target enzymes and transcription factors. They can also serve as hydrogen donors to peroxiredoxins, recently discovered heme free peroxidases, the function of which is to get rid of hydroperoxides in the cell. This review describes the molecular basis for the functioning and interaction between these enzymes in photosynthetic organisms.

Keywords: Disulfide bridge reduction; Redox regulation; Thioredoxin

1. Reactions catalyzed by protein disulfide oxidoreductases

The presence of disulfide bonds is one of the major structural determinants in proteins. It is generally recognized that extracellular proteins possess disulfide bridges that help stabilize their structure, while intracellular enzymes are devoid of such features [1,2]. All polypeptides are however synthesized with free cysteine residues and in the same time that specialized proteins such as chaperones or prolyl isomerases help for the folding process, other enzymes known as protein disulfide isomerases (PDI) are needed for the creation of those disulfide bonds [3]. Conversely, some proteins which contain disulfide bridges need to be reduced to a dithiol form to gain activity and these reactions are catalyzed by thioredoxins (Trx) and other molecular variants of this protein such as glutaredoxins (Grx) [4,5]. PDI, Trx and Grx are thus members of a superfamily whose function is either to create or reduce disulfide bonds on other proteins according to the equation:



2. Molecular and functional characteristics of plant thioredoxins

Plant thioredoxins are small molecular weight (ca. 12 kDa) and generally extremely thermostable proteins. They contain in general around 110 amino acids in their mature form (excluding the transit peptides of the nuclear encoded chloroplastic and mitochondrial isoforms) [6,7]. All thioredoxins have a very conserved active site with the sequence WC[G/P]PC. All available structures demonstrate that the two sulfur atoms of the Cys residues of the active site are indeed linked in a disulfide bridge in the oxidized state. The 3D architecture of the protein is conserved throughout the evolution with a succession of secondary elements as follows: β1, α1, β2, α2, β3, α3, β4, β5, α4. The active site is exposed and situated in the

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Abbreviations: Grx, glutaredoxin; PDI, protein disulfide isomerase; Prx, peroxiredoxin; Trx, thioredoxin.

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loop between the structural units β2 and α2 with the second cysteine integrated in α2 [8–11]. It has been demonstrated in plants as in other organisms that the active site sequence is one major determinant for the efficiency of thioredoxin as a disulfide reductase, with the conserved Trp residue next to the disulfide bridge playing an important role in the process together with a buried conserved Asp residue which exhibits an unusually high pK_a for its carboxyl side chain [12,13]. A comparison of a mean NMR structure together with the crystallographic structure obtained for a wild type *Chlamydomonas reinhardtii* cytosolic thioredoxin is shown in Fig. 1. Similar structural results are obtained using the two techniques, but the secondary elements are more defined in the crystal structure (solid state) than in the NMR one (solution state) due to intermolecular contacts in the crystal state [9]. Interestingly, the helix α1 and strands β4 and β5 are notably longer in the X-ray structure and strand β1 is poorly defined in the NMR structure. In addition, the disulfide bridge is completely integrated in helix α2 in the crystallographic structure, but the catalytic Cys (Cys36 here) is part of the flexible loop in the NMR structure.

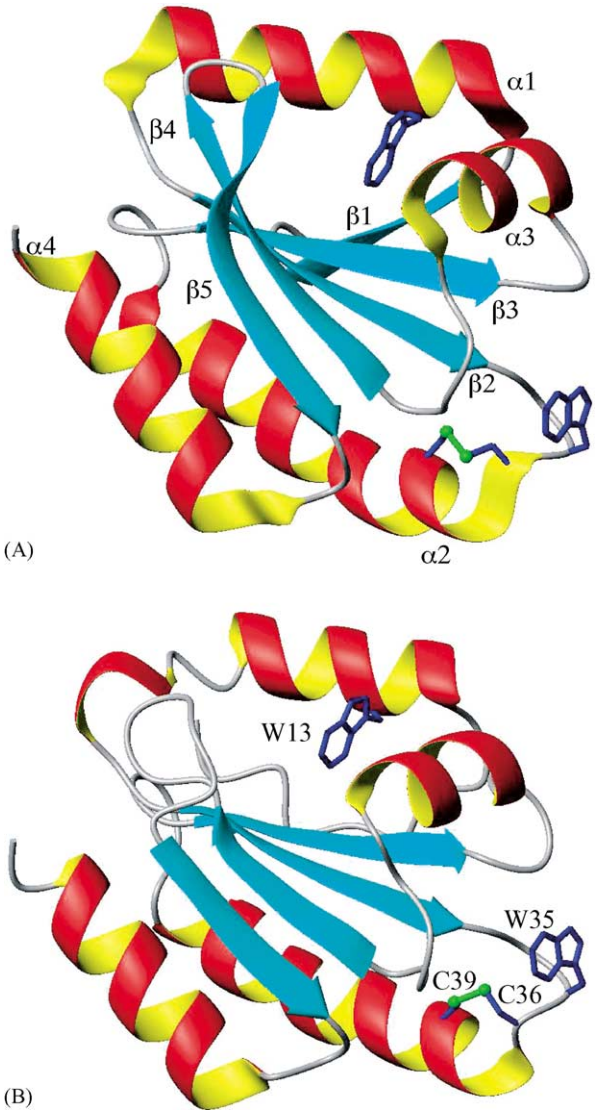

Fig. 1. Comparison of the crystallographic (A) and NMR (B) structures of *Chlamydomonas reinhardtii* thioredoxin h. The protein is shown in the strand and ribbon representation. The disulfide bridge of the active site and the sulfur atoms are in green. The two tryptophane residues (W13 which gives the protein its unique spectral characteristics and W35 adjacent to the active site) are in blue sticks. The figure has been drawn using MolMol (see [9]).

The redox potential of thioredoxin is also critical in governing its reactivity. Plant thioredoxins display redox potentials around −290 mV, a value very similar to the one recorded for thioredoxins in other living organisms [14,15]. Likewise, the pK_a of the thiol groups of the Cys residues of the active site is also an important parameter for disulfide reductase efficiency. NMR titration has shown that the two thiol groups of *C. reinhardtii* thioredoxin h have pK_a's of 7 and 9.5 for the catalytic and backup cysteines, respectively, values similar to those of the bacterial and mammalian enzymes [12,16–18].

A very interesting feature of thioredoxins in plants is the remarkably high number of genes that code for this protein. It has long been assumed that there was a single gene for thioredoxin in *Escherichia coli* and human cells, but recent data have shown that this was not true and that all organisms contain several thioredoxin variants [19,20]. This property has been recognized early in plants, in part because of the discovery of a specific system located in the chloroplasts. With the subsequent discovery that there are also cytosolic and mitochondrial isoforms for this protein, it is now estimated that there is a large multigenic family in plants. For example, the simple model *Arabidopsis thaliana* is estimated to contain probably more than 20 genes overall [21]. This number is likely to be even higher in species with diversified metabolism as the C_4 plant maize, but no serious estimation is available yet in monocotyledonous species.

The high diversity of thioredoxins in plants is accompanied by a diversification in expression and function. It appears that some isoforms are specifically expressed in the chloroplasts, others in mitochondria and some in the cytosol of plant cells [6,7,21]. The expression of some genes seems to be tissue specific and in addition, a remarkable feature in higher plants is that thioredoxin is one major protein constituent of the phloem sap [22,23]. As this tissue permits communication throughout the plant because it is highly connected *via* specialized plasmodesmata, it is likely that thioredoxin plays a role in the long distance signaling. Other possible roles for cytosolic thioredoxins could be the detoxification of heavy metals, the dissipation of oxidative stress and the regulation of transcription factors as in mammalian or yeast cells [24–26]. The mitochondrial thioredoxin system could be involved in the regulation of 2 oxoacid dehydrogenases and alternative oxidase [27,28].

The function of chloroplastic thioredoxins is quite well documented, they serve as redox regulators of specific target enzymes which are generally inactive in the dark

and active in the light, with the notable exception of glucose-6-phosphate dehydrogenase which is oppositely regulated. Through this redox regulation, thioredoxins help control the rate of carbon fixation through the reducing pentose phosphate pathway otherwise known as Calvin cycle [5,29]. Several of the regulatory target enzymes display additional regulatory sequences that contain critical cysteines and these cysteines can be either in a disulfide bond (oxidized form) or as dithiols (reduced state). Generally, the conversion of the oxidized inactive enzymes into reduced active catalysts occurs in two steps, first a fast reduction that produces a reduced inactive enzymes and second a slow conformational change that leads to the active catalyst [30]. The chloroplastic thioredoxin system is unique in at least two ways. First, unlike the mitochondrial and cytosolic systems where the primary reductant is NADPH relayed *via* a flavoprotein NADPH thioredoxin reductase, in chloroplasts the donor is photoreduced ferredoxin through a specific iron–sulfur containing enzyme called ferredoxin thioredoxin reductase [31]. In addition, there are two molecular variants for thioredoxin, named thioredoxin m and thioredoxin f which display differential selectivity. More specifically, thioredoxin f is strictly required for the reductive activation of fructose-1,6-bisphosphatase and other thioredoxins cannot make the job [32]. This is in marked contrast with other systems where thioredoxins are generally considered as a promiscuous enzymes (i.e. they show no marked specificity). Details about the functioning and composition of the regulatory chloroplastic system can be found in several reviews [5,29,33,34]. In chloroplasts, thioredoxins are also involved in the removal of peroxides *via* specific chloroplastic thiol peroxidases or peroxiredoxins (Prx) [35].

3. Glutaredoxins, peroxiredoxins and protein disulfide isomerases share molecular determinants with thioredoxin

The 3D organization of the thioredoxin molecule is well established and referred to as the thioredoxin fold. Based on this architecture, several catalysts have evolved. By keeping basically the same secondary structural units and changing the active site to YCP[Y/F]C, one obtains a glutaredoxin molecule that is still a reductant but with a slightly higher redox potential. Glutaredoxin is reduced *via* a cascade that comprises NADPH, glutathione reductase and the tripeptide glutathione [36,37]. The combination of two thioredoxin modules together with the alteration of the active site sequence to WCGHC leads to protein disulfide isomerase, an oxidizing protein whose function is to create disulfide bonds on other proteins as discussed in the first section [38]. An even more complex version called nucleoredoxin results of the combination of three successive thioredoxin modules, the N- and C-terminus ones bearing each an active site [39]. In addition to these well documented evolutions of thioredoxin, several sequences in the databases feature natural fusion products between a given enzyme (APS reductase or peroxiredoxin for example) together with a glutaredoxin module [40,41]. In addition, many natural monocysteinic variants of thioredoxin and glutaredoxin are present in the protein data banks. These proteins are likely to be catalytically competent as it was demonstrated that engineered monocysteinic glutaredoxins are also functional catalysts [42,43]. Finally, another enzyme which has a fold similar to thioredoxin is peroxiredoxin even though that protein contains a single catalytic cysteine which is transformed into a sulfenic acid upon catalysis [44]. Other examples of structurally related proteins in plants can be found in [5].

4. Functions of thioredoxin related catalysts in plant cells

The function of protein disulfide isomerase is linked to the folding pathway of the ER, PDI sequences containing consistently a KDEL retention sequence that targets them to this compartment [45]. It has been shown however in *C. reinhardtii* that the related protein RB60 which also contains the KDEL signal is targeted both to the ER and to the chloroplast [46]. It has been proposed to play a role as a regulator of chloroplast translational activation [47]. On the other hand, the function of nucleoredoxin is poorly documented but believed to be related to the redox regulation of transcription factors [39]. In plants, glutaredoxins seem to be restricted to the cytosol. This protein has been shown to possess the capacity to reduce dehydroascorbate which could be of physiological significance [43]. Another role proposed for glutaredoxin is to serve as an electron donor to a subclass of peroxiredoxin [48]. In this respect, both the thioredoxin and glutaredoxin pathways could be implicated in the removal of hydroperoxides and the fight against oxidative stress.

5. Future challenges of redox regulation in plants

The high number of genes coding for thioredoxins and related proteins poses the problem of the specificity and possible redundancy of each of these products. This question is starting to be addressed using the double hybrid technique, the yeast and Synechocystis complementation approach and the biochemical isolation of targets. Indeed, the use of engineered monocysteinic mutants has helped isolate targets for given thioredoxin types, but the specificity of this approach needs to be assessed carefully with other parameters such as the putative subcellular localization of the partner proteins [49]. Using the yeast complementation, some specificity is observed between the various isoforms notably concerning the growth on sulfate or methionine sulfoxide and the resistance to hydrogen

peroxide but it is not clear yet that this is of physiological significance [50–53]. Recent attempts to obtain knockout mutants in *Arabidopsis thaliana* by successive PCR screening of transposon transformed pools, have been successful, but generally no clear phenotype appears under physiological conditions. Clearly, sorting out the physiological function for each of these proteins will be a challenge for the years to come.

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