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Review

Thioredoxins and glutaredoxins as facilitators of protein folding

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Abstract

Thiol–disulfide oxidoreductase systems of bacterial cytoplasm and eukaryotic cytosol favor reducing conditions and protein thiol groups, while bacterial periplasm and eukaryotic endoplasmatic reticulum provide oxidizing conditions and a machinery for disulfide bond formation in the secretory pathway. Oxidoreductases of the thioredoxin fold superfamily catalyze steps in oxidative protein folding via protein–protein interactions and covalent catalysis to act as chaperones and isomerases of disulfides to generate a native fold. The active site dithiol/disulfide of thioredoxin fold proteins is CXXC where variations of the residues inside the disulfide ring are known to increase the redox potential like in protein disulfide isomerases. In the catalytic mechanism thioredoxin fold proteins bind to target proteins through conserved backbone–backbone hydrogen bonds and induce conformational changes of the target disulfide followed by nucleophilic attack by the N-terminally located low pK_a Cys residue. This generates a mixed disulfide covalent bond which subsequently is resolved by attack from the C-terminally located Cys residue. This review will focus on two members of the thioredoxin superfamily of proteins known to be crucial for maintaining a reduced intracellular redox state, thioredoxin and glutaredoxin, and their potential functions as facilitators and regulators of protein folding and chaperone activity.

Keywords: Thioredoxin; Glutaredoxin; Thiol–disulfide; Protein disulfide; Redox potential

1. Introduction

From bacteria to eukaryotic cells, cellular compartmentalization provides well defined reaction chambers for the variety of vital biochemical processes. One of the most distinguishing features of the different compartments is their redox potential. In general, the redox potential describes the tendency of a system to either gain or lose electrons when new species are introduced. Compartments with low redox potential will rather reduce newly introduced compounds, while compartments with high redox potential oxidize them. In this respect, protein cysteinyl side chains are of particular interest, because their thiol group (P-SH) is susceptible to a number of oxidative modifications. The reversible formation of inter- or intramolecular disulfides (P-S-S-P) is frequently a prerequisite for the proper folding and function of a protein or protein complex. Moreover, disulfide bond formation as well as the reversible formation of protein glutathione (GSH) mixed disulfides (glutathionylation) and *S*-nitrosylation of protein thiols act as regulatory switches modulating the activity of numerous proteins in response to alterations in the cellular redox state. Severe oxidative stress, i.e. the excessive formation of reactive oxygen species, may lead to the formation of sulfenic (P-SOH), sulfinic (P-SO_2H) and sulfonic (P-SO_3H) acid. The redox potential of the bacterial cytoplasm as well as the eukaryotic cytosol normally favors reduced thiol groups, while the bacterial periplasm and the eukaryotic endoplasmatic reticulum provide the oxidizing environments for disulfide bond formation in proteins to be exported from the cell or to the cell surface. In biological systems, thiol–disulfide exchange reactions are highly specific with respect to the thiol groups involved. This specificity is the result of the unique physico-chemical micro-environment of susceptible thiol groups and of enzymatic catalysis by a group of oxidoreductases that belong to the thioredoxin family of proteins.

2. The thioredoxin family of proteins

Thioredoxin (Trx), the eponymous founder of the thioredoxin family of proteins, was first identified as electron donor

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for ribonucleotide reductase (RNR) from *E. coli* [1,2]. In all organisms, this group of enzymes is central for DNA synthesis both during replication and repair [3]. The second member of the Trx family, glutaredoxin (Grx), was discovered as a GSH-dependent electron donor for RNR in an *E. coli* mutant lacking Trx [4]. Today, the Trx protein family is first and foremost defined by a structural motif named the Trx fold [5]. This fold is common to a variety of functionally different proteins [6,7], for instance, thiol–disulfide oxidoreductases, disulfide isomerases, glutathione *S*-transferases [8], thiol dependent peroxidases [9], and chloride intracellular channels [10].

2.1. The thioredoxin fold

The Trx fold motif consists of a central four-stranded β-sheet surrounded by three α-helices [11,12]. This basic structure can only be found in bacterial glutaredoxins, while thioredoxins contain an additional β-sheet and α-helix at the N-terminus [13]. Variations of this motif (Fig. 1) have also been identified in the Trx fold domains of *E. coli* DsbA, a protein necessary for disulfide bond formation [14], protein disulfide isomerases (PDI) from human and yeast [15,16], and PDI homologous proteins DsbC and DsbG from *E. coli* [17,18]. Noteworthy, the Trx domain in these proteins is so well conserved that it can be used to identify novel members of the family, as shown for human PDIs [19].

Hallmarks of the Trx motif are a *cis*-proline residue located before β-sheet three and the Cys-X-X-Cys active site motif located on the loop connecting β-sheet one and α-helix one [11]. The question of why some of these structurally similar proteins act as reductants while others are more efficient oxidases was the subject of several investigations. These studies revealed a major role of the two residues in between the two active site cysteines. Even though other residues have been described to affect the redox potential [20–22], the primary focus of these studies has been on the amino acids in the active sites. The nature and composition of these two amino acids dramatically affects the standard redox potential of the particular proteins. In *E. coli*, the strongest reductant, cytosolic Trx (Cys-Gly-Pro-Cys), has a redox potential of $\Delta E'_0 = -270$ mV [23], the strongest oxidant, periplasmatic DsbA (Cys-Pro-His-Cys), has a redox potential of $\Delta E'_0 = -122$ mV [24]. The redox potential of human PDI (Cys-Gly-His-Cys) was determined to be $\Delta E'_0 = -175$ mV [25]. Mutation of the Cys-Gly-Pro-Cys active site in Trx to the corresponding Cys-Gly-His-Cys active site of PDI resulted in an increase of its standard midpoint potential to $\Delta E'_0 = -235$ mV and $\Delta E'_0 = -221$ mV, respectively [23,26]. This change in redox potential was accompanied by a 10-fold increase in PDI activity [23,27]. Exchange of Trx's active site for the Cys-Pro-His-Cys motif found in DsbA proteins increased the redox potential further to $\Delta E'_0 = -204$ mV [26]. Corroboratively, the redox potential of a DsbA mutant harboring the Cys-Gly-Pro-Cys active site of Trx decreased by 92 mV [28].

2.2. The thioredoxin system

Trx utilizes the two cysteinyl residues in its Cys-Gly-Pro-Cys active site to reduce the protein disulfide formed during the

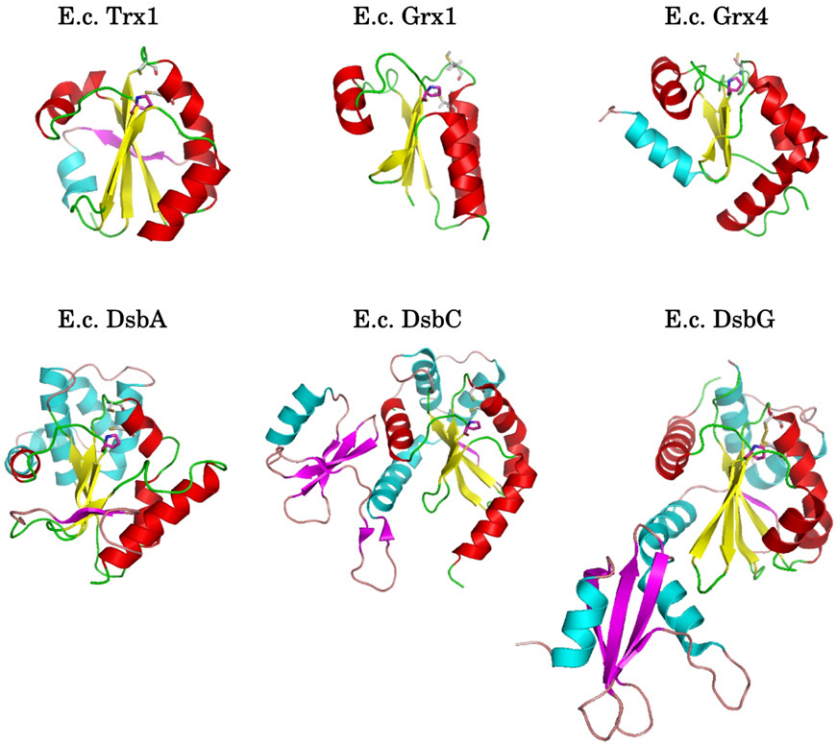

Fig. 1. Selected structures of thioredoxin (Trx) fold oxidoreductases from *E. coli*. The secondary structural elements of the Trx fold are shown in yellow and red, additional elements are shown in magenta and blue. The *cis*-Pro residues are depicted in purple, the active site Cys residues in grey. Trx1 (PDB entry: 1xob), dithiol glutaredoxin 1 (1egr), monothiol glutaredoxin 4 (1yka), DsbA (1a2l), DsbC (1tjd), and DsbG (1v58).

catalytic cycle of the RNR. Since 1979 we know Trx as the general disulfide reductase [29] reducing disulfide bonds by a ping-pong mechanism [30]. Recently, it was shown by single molecule force-clamp spectroscopy that efficient catalysis requires a reorientation of the substrate disulfide bond [31]. This investigation demonstrated, that the rate limiting step of Trx activity is the orientation of the N-terminal active site cysteine of Trx and the two disulfide bridged cysteines of the substrate in a 180° angle (Fig. 2, 1). The resulting shortening of the overall length of the substrate polypeptide by around 0.8 Å resulting in a shortened projection of the disulfide bond is the condition for the nucleophilic attack of the N-terminal Cys [31]. The N-terminal active site thiol of Trxs possesses an unusual low pK_a value [32]. Hence, the thiol group of this cysteinyl side chain is readily deprotonated under physiological conditions. The resulting thiolate acts as nucleophile attacking the target disulfide bond to form a covalent intermediate mixed disulfide between the Trx N-terminal active site and one of the substrate's cysteinyl side chains [32] (Fig. 2, 2). This disulfide is subsequently reduced by the C-terminal active site cysteinyl side chain yielding the reduced substrate and a disulfide in the active site of Trx (Fig. 2, 3). Subsequently, the disulfide in the active site of Trx is reduced by the dimeric flavo-enzyme thioredoxin reductase (TrxR) at the expense of NADPH (for a more detailed overview, see Refs. [12] and [33]) or by ferredoxin via ferredoxin-dependent TR in chloroplasts [34].

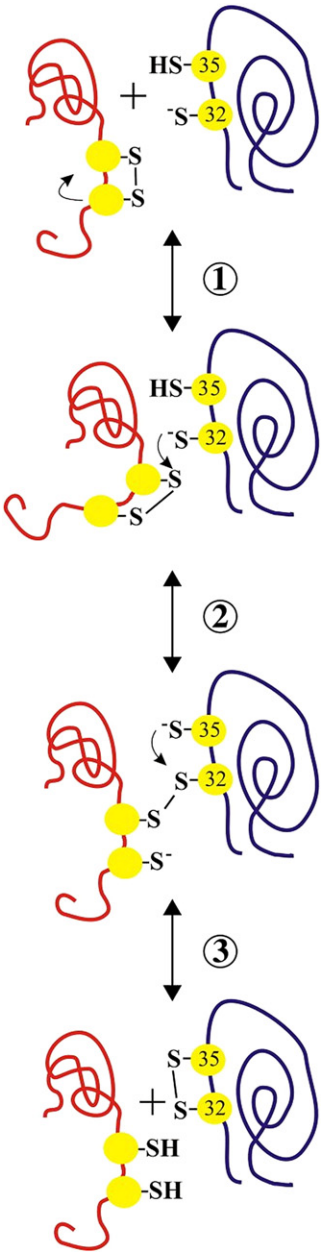

Fig. 2. Interaction of thioredoxin (Trx) with proteins. Trx catalyzes reversibly the reduction of disulfides via a covalent-mixed disulfide intermediate (see text). The direction of the reaction depends on the redox potential of the environment. Blue: human Trx1, red: substrate.

Trxs (around 10–12 kDa) are ubiquitous proteins present in all forms of life. *E. coli*, yeast and mammalian cells contain two Trxs each [35]. The mammalian proteins are localized in the cytosol/nucleus (Trx1) and in mitochondria (Trx2) [36]. Plants contain a rich variety of Trxs with sometimes highly specialized functions [34,37,38]. Trxs are crucial proteins for oxidative stress defense, redox regulation of cellular function, and different metabolic pathways (for detailed overviews: [5,39–42]). For instance, Trxs are essential cofactors for DNA synthesis [1,43], sulfur assimilation [44], and peroxiredoxins [45]. Trxs regulate the activity of several transcription factors, such as NF-κB in mammals [46], and metabolic enzymes such as fructose-1,6-bisphoshatase in chloroplasts [47]. The importance of Trxs in several aspects of human health, e.g. pregnancy, embryonic development, cardiovascular and neurodegenerative diseases, or virus infection was object of a recent review [42]. In the scope of this review, it is important to note that a number of functions described for Trxs are independent from their oxidoreductase activity, for instance *E. coli* Trx as subunit of the T7 DNA polymerase complex [48], the redox regulation of apoptosis signaling kinase 1 by human Trx [49], and its activity as molecular (co-)chaperone (see below).

2.3. The glutaredoxin system

Glutaredoxins (Grxs) are small proteins, usually around 9–15 kDa, existing in large number of isoforms in basically all glutathione (GSH)-containing life forms. Based on their active site motifs, Grxs can be divided in two major categories: the dithiol Grxs (active site Cys-Pro-Tyr-Cys) and the monothiol Grxs (active site: Cys-Gly-Phe-Ser).

Dithiol Grxs are general thiol–disulfide oxidoreductases with high specificity for the GSH moiety. These Grxs are kept reduced by GSH. The resulting glutathione disulfide (GSSG) is reduced by glutathione reductase (GR) with electrons from NADPH [50,51]. The classical dithiol Grxs efficiently reduce some protein disulfides like that in *E. coli* ribonucleotide reductase with a mechanism as described for Trxs above (dithiol mechanism, Fig. 3A). However, the major action of Grxs seems to be to reduce protein–GSH mixed disulfides (de-glutathionylation) utilizing a mechanism that requires only the N-terminal active site residue (monothiol mechanism, Fig. 3B, for a more detailed overview, see reference [33]. *E. coli* contains three dithiol glutaredoxins [52,53], yeast and mammalian cells

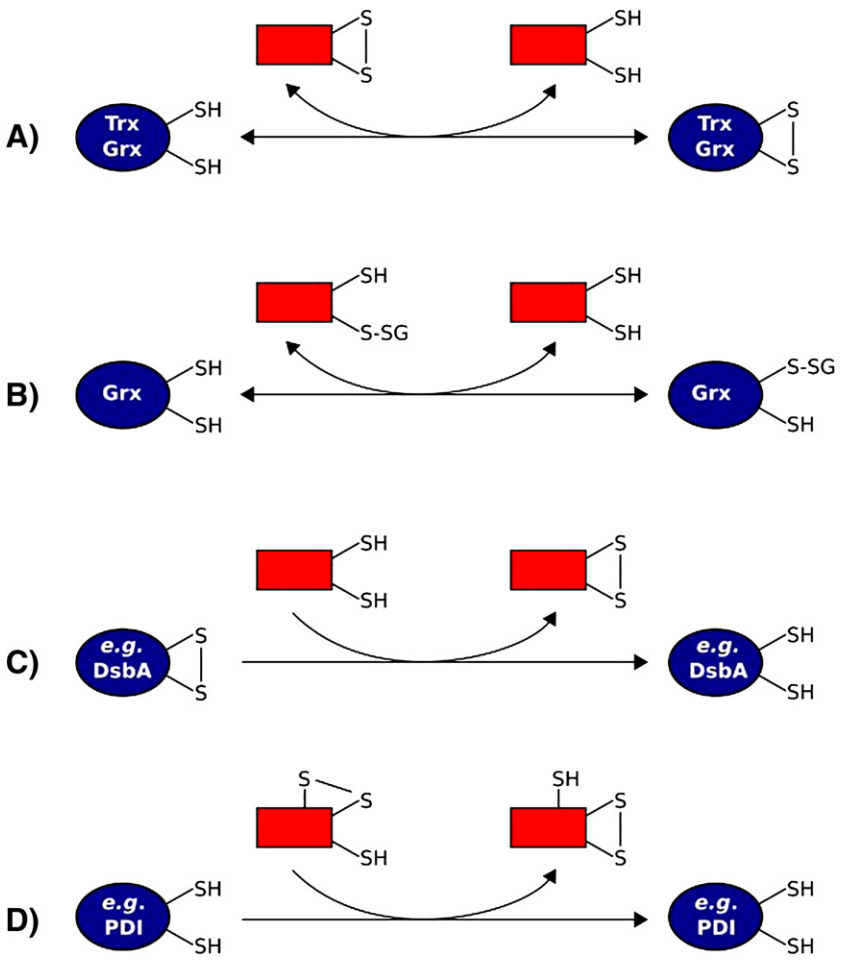

Fig. 3. Catalyzed reactions by oxidoreductases of the thioredoxin family of proteins. A) Reduction of intramolecular disulfides, B) reduction of protein–GSH mixed disulfides, C) oxidation of thiol groups, formation of disulfides, and D) isomerization of disulfides.

contain two, one in the cytosol, one in mitochondria [54,55,56,57]. The situation in plants is far more complex, the genome of *Arabidopsis thaliana*, for instance, encodes 14 dithiol Grxs [58].

Numerous functions have been described for Grxs, both as electron donors as well as regulators of cellular function in response to oxidative stress, for instance, in sulfur assimilation [59,60], dehydroascorbate reduction [61] and the regulation of cellular differentiation [62], transcription [63–65], and apoptosis [66–68]. Human glutaredoxins have been implied in several diseases [42,33]. Recently, some dithiol glutaredoxins have been described as iron–sulfur proteins. Human mitochondrial Grx2 and poplar GrxC1 were described as the first members of the thioredoxin family of proteins coordinating an [2Fe2S] cluster [69,70]. The clusters are coordinated by the N-terminal active site cysteine of two Grx monomers and two non-covalently bound molecules of GSH [69,71]. Both human Grx2 and poplar GrxC1 differ in their active site from the dithiol Grx consensus sequence Cys-Pro-Tyr-Cys. Grx2 contains a serine, GrxC1 a glycine instead of the active site proline residue. This exchange provides enough flexibility and space for the non-covalent binding of GSH and cluster coordination. In fact, when the active site of other Cys-Pro-Tyr-Cys Grxs, for instance human Grx1 which normally cannot bind the cluster, are changed to Cys-Ser-Tyr-Cys or Cys-Gly-Tyr-Cys, these proteins become able to complex an iron–sulfur cluster [70,71]. In all other members of the thioredoxin family containing a Cys-X-X-Cys active site the conserved *cis*-proline was suggested to preclude metal binding [72].

The molecular mechanism and the functions of monothiol glutaredoxins are only beginning to emerge (for a recent review see [73]). Noteworthy, most monothiol Grxs are essentially inactive in (dithiol) Grx-specific activity assays. Monothiol Grxs from *E. coli*, yeast, and vertebrates including humans are crucially involved in iron–sulfur cluster biosynthesis and regulation of iron homeostasis [74–79]. *E. coli* contains one monothiol Grx [80], yeast five [81,82], and mammals two [78,83]. Similar to dithiol Grxs, plants contain a rich variety of isoforms, e.g. at least 17 different monothiol Grxs are encoded in the genome of *A. thaliana* [58].

3. Glutaredoxins and thioredoxins as facilitators of protein folding

Trxs are by far the most well known members of the Trx family. The investigations on these proteins, as well as Grxs, provided general insights in functions and mechanisms of all

members of the Trx family. Even though most of these initial general experiments were done in vitro, they provided important concepts for the in vivo situation. Trxs and Grxs can be involved in protein folding in many different ways (Fig. 4). The list of proteins interacting with Trxs and Grxs is growing continuously. The reduction of protein disulfides includes and often results in, sometimes subtle, structural changes of the target protein [31,12,84,85]. But Trxs and Grxs do not necessarily act as disulfide reductases, under certain conditions these redoxins promote disulfide bond formation and synergistically work with PDI and/or chaperones.

3.1. Protein disulfide isomerization

PDIs catalyze the isomerization of disulfide bond formation in the oxidative environments of the prokaryotic periplasm and the eukaryotic endoplasmatic reticulum (Fig. 3D, overviews in [86,87], and several contributions to this special issue of Biochimica et Biophysica Acta). The investigation of an in vitro model of the mechanism by which PDI catalyzes formation of disulfide bonds in presence of GSSG indicated that PDI primarily catalyzes formation and breakage of GSH–protein mixed disulfides [88]. For this function, only the N-terminal cysteine is essential as shown by kinetic analyses of ribonuclease A (RNase A) refolding [89]. As described above, Grxs are GSH mixed disulfide reductases. Therefore the effect of Grx in GSH-dependent refolding of reduced RNase A was investigated. *E. coli* Grx1 displayed synergistic activity together with PDI when the redox potential of the GSH redox buffer was low enough to reduce the active site disulfide in Grx [90]. This synergistic effect can be explained by the fact that Grx (i) converted formed mixed disulfides into intramolecular disulfide bonds in the presence of PDI and (ii) was able to catalyze both the formation and the reduction of GSH mixed disulfides [91]. For this last reason *E. coli* Grx1 was also able to refold RNase A without PDI [91]. More recently, it was demonstrated that oxidized *E. coli* Grx1 catalyzes refolding – in concert with GSH – around 30 fold more efficient than PDI [92]. The further investigation of the mechanism showed that Grx1 acted by the monothiol mechanism (Fig. 3B). Only Cys11 was involved in this activity indicating ribonuclease–GSH mixed disulfides as substrate [90].

Therefore, GSH mixed disulfides were believed to be important folding intermediates in vivo. Moreover, the relative amount of oxidized GSH in the ER is around 30 times higher than in the cytoplasm [93] and the ratio of reduced and oxidized GSH is optimal for disulfide bond formation [94]. Today it seems, that in eukaryotic systems GSH is needed as a redox balancing system, providing reducing equivalents for the reduction of PDIs, and for the protection against reactive oxygen species, which are formed during oxidation of PDIs via Ero1 using oxygen as the ultimate electron acceptor (reviewed in: [95]).

Already back in 1986 it was shown that oxidized and a mixture of oxidized and reduced Trx efficiently catalyzes the refolding of reduced, denatured RNase, or oxidized, scrambled RNase, respectively [96].

In *E. coli*, oxidative folding of proteins takes place in the periplasm. The two PDIs DsbC and DsbG reduce incorrectly formed disulfide bonds for further isomerization [86]. A constant electron supply is guaranteed by the membrane protein DsbD which itself is reduced by cytoplasmic Trx [97]. Thus, electrons provided by Trx are essential for the correct oxidative folding of proteins in the *E. coli* periplasm.

Moreover, Trx, when exported to the periplasm, is able to complement *E. coli* strains deficient in the periplasmatic thiol oxidase DsbA at concentrations that allow efficient re-oxidation of Trx by DsbB [98]. As outlined before, Trx mutants mimicking the active sites of other Trx fold oxidoreductases result in a higher redox potential and increased PDI activity [23,99,26]. Corroboratively, these mutants were also more efficient in complementation of DsbA deficiency in the periplasm [100,98]. Masip et al. [101] aimed at engineering a disulfide bond formation pathway independent of DsbA and DsbB. To achieve this goal, active site mutants of *E. coli* Trx1 were created by random mutagenesis and tested for the ability to complement a DsbA/DsbB deficient *E. coli* strain. Surprisingly, the most efficient mutants contained the active sites Cys-Ala-Cys-Cys and Cys-Ala-Cys-Ala and coordinated an [2Fe2S] cluster that was essential for the catalysis of oxidative protein folding.

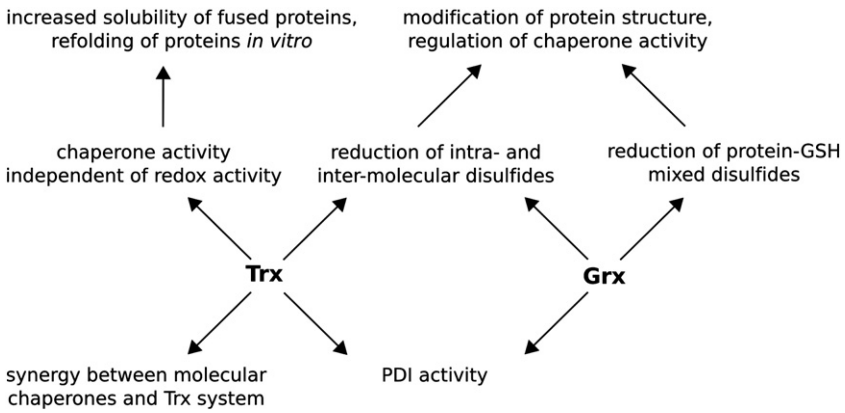

Fig. 4. Thioredoxin (Trx) and glutaredoxin (Grx) as facilitators and regulators of protein folding (for details see text).

Trxs and Grxs are also involved in disulfide bond formation in the cytoplasm. Usually these proteins maintain a reducing environment in the cytosol by reduction of disulfide bonds. However, when the Trx system is impaired, for instance by inhibition of TrxR, disulfide bond formation can occur in the cytoplasm of *E. coli* [102]. This effect is enhanced if, in addition to TrxR, GR and thereby the Grx system is disturbed [103]. This formation of disulfide bridges in the cytoplasm of *E. coli* is not only attributed to the lack of reductase activity of Trxs and Grxs, but also to the accumulation of oxidized redoxins that serve as oxidants and catalysts of disulfide bond formation [104].

3.2. Chaperone activity and interactions

Besides their disulfide formation and isomerization activity, thioredoxins and related oxidoreductases have been shown to promote the folding of proteins independent of their redox activity both by directly promoting protein folding and by enhancing the refolding activity of other molecular chaperones.

As described above, the overall structure of the substrate is shortened involving a conformational change of the polypeptide during binding to Trx [31] (Fig. 2, 1). Hence, chaperone activity is coupled to rate enhancements in the order of four to five orders of magnitude compared to small reductants like dithiothreitol in reduction of protein disulfides.

Thioredoxin fusion proteins have become a widely used tool to avoid inclusion body formation during recombinant expression of proteins in *E. coli*. Several plasmids were constructed for expression of these fusion proteins, for instance [105,106]. Trx fusion markedly increased the levels of soluble proteins heterologously expressed in *E. coli*, for instance numerous mammalian proteins [105–107], *Clostridium tetani* fragment C of tetanus toxin [108], or the single chain variable fragment of antibodies [109]. The induction of correct folding was independent of Trx's redox activity since fusion with a Trx mutant harboring an Ala-Gly-Pro-Ala "active site" was as efficient as the wild type Trx fusion partner [109]. Trx, covalently linked to the protein of interest, may act as molecular chaperone preventing precipitation and aggregation of the fused partners until these reach a stable folding state [105,106]. Moreover, in cases where Trx fusion failed to increase solubility of target proteins, it increased the yield of soluble protein following solubilization and refolding from inclusion bodies [110]. In vitro, *Helicobacter pylori* Trx1, but not Trx2, promoted the renaturation of arginase [111]. *E. coli* Trx1 and the Trx homologue YbbN/Trxsc were able to refold citrate synthase and α-glucosidase with an efficiency comparable to those of chaperones like DnaK and different heat shock proteins [112,113]. Remarkably, neither the redox state of the Trxs nor the active site cysteines or other amino acids important for redox function are required for chaperone activity [112,111]. Moreover, Trx from *E. coli* can stimulate the refolding of MglB, a protein without cysteines [112]. Even though Trx and YbbN/Trxsc promote the renaturation of citrate synthase, they do not protect citrate synthase against thermal degradation. Interestingly, TrxR from *E. coli* protects citrate synthase as efficient as DnaK from thermal degradation [112,113]. Unlike molecular chaperones, TrxR, Trx and YbbN/Trxsc do not preferentially bind unfolded proteins [112,113].

Eukaryotic mitochondrial monothiol Grx5 was implied in iron–sulfur cluster biosynthesis [74,75]. Depletion of Grx5 led to increased iron levels and decreased enzymatic function of iron–sulfur proteins such as aconitase and succinate dehydrogenase [74]. In eukaryotes iron–sulfur cluster biosynthesis takes place in mitochondria. The clusters are first assembled on scaffold proteins and subsequently transferred to their target proteins (reviewed in [114]). This transfer is dependent on a functional HscA/HscB chaperone system [115]. The phenotype of Grx5 deletion in yeast can be rescued by overexpression of the HscA-type chaperone Ssq1 [74]. Biochemical data from Mühlenhoff et al. [75], showing that deletion of Grx5 resulted in accumulation of iron–sulfur clusters on the scaffold protein, supports the model that Grx5 may act in concert with the HscA/Ssq1 HscB/Jac1 chaperone couple in the transfer of the iron–sulfur cofactor from the scaffold to the target protein.

Several proteomic approaches aiming at the identification of new targets of the Trx and Grx systems indicate interactions between these proteins and molecular chaperones. Human Trx1 was identified as interaction partner of 14-3-3… proteins during interphase and mitosis in HeLa cells [116]. Using poplar Grx-C4 as bait and several plant extracts as samples Rouhier et al. identified a 14-3-3 protein (At5g6543), Hsp60, Hsp70 and cyclophilin as interacting proteins [117]. Cyclophilin was also found in investigations aiming at the identification of new targets of *A. thaliana* cytosolic Trx-h3 and spinach plastidic Trx-m [118,119]. Cyclophilin, a peptidyl-prolyl *cis–trans* isomerase essential for protein folding [120,121] is activated via reduction by spinach Trx-m [122]. Synergistically with α-crystallin, the Trx system can recover inactivated GR in human aged clear and cataract lenses [123]. The Trx system is known to interact with peroxiredoxins (Prxs), some of which were characterized as chaperones. The yeast cytosolic Prxs 1 and 2 and the human Prx2 interact with unfolded substrates and do suppress thermal degradation of citrate synthase [124,125]. Human Prx1 protects malate dehydrogenase against heat-induced denaturation [126]. Like other chaperones yeast Prx1 forms temperature-dependent multimers, thus increasing chaperone activity and decreasing peroxidase activity [124]. As for Trx, this chaperone activity was independent of the conserved active site cysteines [124]. In contrast, H_2O_2 induced chaperone activity of yeast and human Prxs 2 requires the active site cysteinyl side chains and the Trx system as cofactor [124,125]. In concert with yeast Prx2, the Trx system protects ribosomes against stress-induced aggregation [127].

Several chaperones have been characterized to be redox regulated by reversible oxidation of thiol groups [128,129]. Trx and Grx catalyze the reduction of protein disulfides in *E. coli* Hsp33 [130], yeast Ssp2 [131], and human Hdj2 [132]. Proteomic approaches identified the following chaperones as being regulated by reversible glutathionylation: Hsp10, [133], Hsp60 [134,135], Hsp70 [134–136,133], Hsp90 [134], Hsc70 [134,136], 14-3-3 proteins [134], and cyclophilin A [135,136,133]. Reversible glutathionylation was also described as negative regulatory mechanism for human Cox17 activity [137]. Grxs are highly specific for protein–GSH mixed disulfides. Hence, all the above mentioned

chaperone–GSH mixed disulfides are likely targets for Grxs in vivo. In fact, glutathionylated human Hsc70 was characterized as substrate for Grx1, reduction of the mixed disulfide decreased chaperone activity [138].

4. Concluding remarks

A wealth of results shows that Trxs and Grxs participate at multiple stages in protein folding. New thioredoxins and glutaredoxins, such as the emerging group of monothiol Grxs, have already been implied in protein maturation. Members of the thioredoxin superfamily of proteins show a general preference for protein–protein interactions, employing the Trx fold as universal interaction scaffold. The different systems are not in thermodynamic equilibrium but kinetic factors and the distribution of the thioredoxin superfamily members in compartments and associated NADPH dependent enzymes and GSH and GSSG concentrations determine have to be taken in account. It can be expected that future research will yield more functions of thioredoxins and glutaredoxins and related proteins as facilitators and regulators of protein folding.

Acknowledgments

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