

Thioredoxin and Glutaredoxin Systems*

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Thioredoxin is known as a hydrogen donor for ribonucleotide reductase, the essential enzyme providing deoxyribonucleotides for DNA replication (1-4). Characterization of viable *Escherichia coli* mutants lacking thioredoxin (1, 5) resulted in the discovery of glutaredoxin and glutathione as a hydrogen donor for ribonucleotide reductase calling into question the role of thioredoxin (6). Thioredoxin and glutaredoxin are small proteins containing an active site with a redox-active disulfide; they function in electron transfer via a simple and elegant mechanism, the reversible oxidation of two vicinal protein-SH groups to a disulfide bridge. Both can supply ribonucleotide reduction and other reactions with electrons from NADPH via their specific reduction mechanism (Fig. 1). Thus, the thioredoxin system is composed of NADPH, the flavoprotein thioredoxin reductase, and thioredoxin; the glutaredoxin system of NADPH, the flavoprotein glutathione reductase, glutathione, and glutaredoxin. Research on thioredoxin and glutaredoxin in many organisms has revealed a surprising variety of different functions. This may reflect the general importance of sulfhydryl groups and disulfides in biochemical mechanisms and cellular regulation plus the adaptation and use of an early structure during evolution of new physiological functions. This minireview will focus on certain aspects of what we know and would like to know regarding thioredoxin and glutaredoxin systems. The reader is referred to other reviews (1, 7, 8) for a more comprehensive treatment of the subject.

Electron Transport to Reductive Enzymes

Cells require ribonucleotide reduction for DNA synthesis, sulfate reduction (9) to generate reduced sulfur of *e.g.* cysteine, and reduction of methionine sulfoxide (10) either as the free amino acid or for the repair of oxidatively damaged side chains in proteins. These reactions are catalyzed by specific substrate reductases that share a requirement of electrons from NADPH via -SH groups from the thioredoxin or glutaredoxin systems (Fig. 1). It is assumed that these enzymes also contain redox-active cysteine residues (11) as part of their active sites.

Ribonucleotide Reductase—This important enzyme is responsible for reduction of all four ribonucleotides to the corresponding deoxyribonucleotides (2, 3); enzyme activity and specificity are controlled by allosteric effectors and are expressed only in replicating cells (4). The enzyme from *E. coli* is similar to that from mammalian cells and is composed of two homodimeric different subunits (B1 and B2). Ribonucleotide reductase is separated from its natural hydrogen donor(s) during purification. Assays of the enzyme in cell-free extracts are complicated by subunit dissociation and side reactions causing a low observed activity, often a fraction of what theoretically is required for DNA replication (2). The enzyme uses a free radical mechanism plus internal -SH groups that are oxidized to a disulfide during reduction of the 2'-OH of the ribose moiety (4, 8, 11). Turnover requires reduction by a dithiol. Both thioredoxin and glutaredoxin (12, 13) can satisfy this requirement *in vitro* but exhibit distinct properties. In *E. coli*, thioredoxin is abundant (~10,000 copies/cell) and shows a comparatively high apparent K_m value for ribonucleotide reductase (1.3 μM). Stated otherwise, it behaves like a substrate with a lower turnover number than the B1 and B2 subunits of the enzyme (13). Mechanistically, thioredoxin operates by a ping-pong mechanism with both thioredoxin reductase and ribonucleotide reductase

(1, 2). Binding, dissociation, and diffusion obviously lower the turnover number.

E. coli glutaredoxin is normally much less abundant than thioredoxin (9) but highly variable for undetermined reasons (8, 14). Glutaredoxin is a general GSH-disulfide-oxidoreductase (or transhydrogenase); *E. coli* is a rich source of such additional uncharacterized activities (12). The apparent K_m value of glutaredoxin with ribonucleotide reductase is lower than that for thioredoxin (~10-fold); the corresponding turnover number is similar or higher than that for ribonucleotide reductase (13). Mechanistically, glutaredoxin thus behaves like a subunit of ribonucleotide reductase with GSH as a diffusible cofactor (13). Glutathione in cells (reviewed in Ref. 15) is usually almost completely in the reduced form and present in high concentration (1-10 mM). Glutaredoxin- and GSH-dependent ribonucleotide reduction is highly sensitive to inhibition by the product GSSG (13). In the absence of a reducing agent and under aerobic conditions, ribonucleotide reductase becomes inactive, probably by formation of additional structural disulfides. Glutaredoxin shows no activity with such preparations of the enzyme (13), but thioredoxin can restore activity by acting as a general protein disulfide reductase; in this way it may regulate the enzyme by thiol redox control (1).

Is thioredoxin or glutaredoxin the hydrogen donor for ribonucleotide reductase *in vivo*? This question has been examined in *E. coli* using mutants (13). Removal of any of the individual components of the thioredoxin or the glutaredoxin systems (Fig. 1) still permits growth, even on minimal media (1, 14, 16); this includes mutants lacking either thioredoxin (*trxA*) (1) or glutaredoxin (*grx*) (17). Attempts to construct a double mutant lacking both thioredoxin and glutaredoxin by P1 transduction of a *grx::kan* gene into a ΔtrxA strain gave unexpected results (17); the desired mutant called A410 that lacked both thioredoxin and glutaredoxin (17) was viable but required cysteine for growth on a minimal medium. This can be explained by the role of thioredoxin and glutaredoxin in sulfate reduction as a hydrogen donor for adenosine 3'-phosphate 5'-phosphosulfate reductase (18) (Fig. 1). Further analyses show that thioredoxin or glutaredoxin is essential for sulfate reduction but apparently not for deoxyribonucleotide synthesis.¹ This strongly indicates the existence of a third unknown hydrogen donor for ribonucleotide reduction¹; the properties of such a system are so far unknown. Also, the simple picture assuming one essential ribonucleotide reductase in *E. coli* (2, 4) has recently been upset by the discovery of a novel, anaerobic, and oxygen-sensitive ribonucleotide reductase activity (19, 20). Immunohistochemical investigations of *E. coli* using specific antibodies showed high concentrations of glutaredoxin exclusively in restricted areas or mesosome-like structures at the cell membrane; thioredoxin is either located at the cell membrane or in the nucleoid region (1). The localization of ribonucleotide reductase is unknown. Usually thioredoxin is active with ribonucleotide reductase from other species, but neither the C-1 thioredoxin of *Corynebacterium nephridii* (21) nor the thioredoxin of rabbit bone marrow (22) is a hydrogen donor for the homologous ribonucleotide reductase *in vitro*. Glutaredoxin shows species specificity indicating coevolution with ribonucleotide reductase (1, 16). Clearly, much more has to be done yet to understand the role of thioredoxin and glutaredoxin in ribonucleotide reduction and the ways cells have developed to afford protection for this essential metabolic pathway.

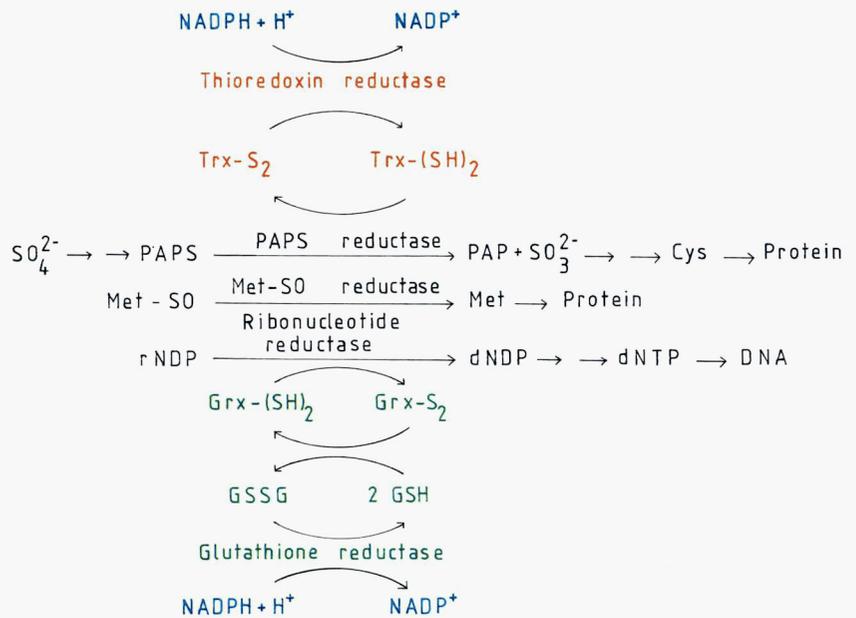
Thioredoxin in Virus Life Cycles

Thioredoxin or glutaredoxin plays a role in the host-virus relationship and life cycle of three *E. coli* phages of widely different complexity, namely M13, T7, and T4. Thus, thioredoxin is essential for the assembly of the filamentous phages f1 and M13, originally defined as the *tip* gene product (23). This role of thioredoxin, at the cell membrane, requires the reduced form and therefore also partly the activity of thioredoxin reductase since thioredoxin-S₂ is inactive (24). Most likely, thioredoxin interacts with the gene I product of f1 or M13; the

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FIG. 1. Outline of thioredoxin and glutaredoxin system and role in cellular reductions in *E. coli*. PAP, phosphoadenosine 3'-phosphate; PAPS, adenosine 3'-phosphate 5'-phosphosulfate; *Trx-S₂*, oxidized thioredoxin; *Trx-(SH)₂*, reduced thioredoxin; *Grx-S₂*, oxidized glutaredoxin; *Grx-(SH)₂*, reduced glutaredoxin.



understanding of the role played by thioredoxin in this process is limited, but no redox cycle is required (24). This has been shown by site-directed mutagenesis of thioredoxin, where Cys-32 and Cys-35 of the active site have been replaced by serine residues without a change in activity *in vivo* (23). A general role of thioredoxin to promote secretory processes at membranes actually fits the pictures of the cellular distribution of thioredoxin obtained by immunohistochemical methods using *E. coli* or mammalian cells (see below) (1).

Phage T7, a medium sized virus, incorporates *E. coli* thioredoxin as an essential subunit of its phage-encoded DNA polymerase (5, 25). T7 DNA polymerase is a stable ($K_d \sim 5$ nM) noncovalent 1:1 complex of thioredoxin-(SH)₂ (12 KDa) and the T7 gene 5 protein (80 KDa) (25). The enzyme has been purified to homogeneity by antithioredoxin immunoadsorbent chromatography (26). The gene 5 protein has DNA polymerase activity although with low processivity and turnover. Binding of thioredoxin stimulates activity about 100-fold and gives the enzyme a very high processivity (25). Only thioredoxin-(SH)₂ activates the gene 5 protein; thioredoxin-S₂ is inactive (27). As with f1 or M13, no redox cycle is involved, and a mutant thioredoxin with both active site half-cystines (Cys-32 and Cys-35) replaced by serine residues can give the same maximal DNA polymerase activity; however, the mutant protein binds with about a 100-fold lower affinity to the gene 5 protein (27).

What can we learn from the role of thioredoxin in T7 DNA polymerase? So far, no analogous role of thioredoxin in *E. coli* DNA replication has been found (28). However, mutations in the gene for *dnaA*, involved in initiation, can be suppressed by mutations within the *trxA* gene (29).

Understanding the mechanistic role of thioredoxin in T7 DNA polymerase will require an x-ray crystallographic structure of the enzyme, not yet achieved. Recently, T7 DNA polymerase with low exonuclease activity has been successfully applied as a DNA sequencing enzyme with the dideoxy method (30).

The large T4 phage uses a virally encoded T4 ribonucleotide reductase together with a T4 thioredoxin to synthesize deoxyribonucleotides for virus replication in the infected *E. coli* cell (2). T4 thioredoxin is a substrate for the host *E. coli* thioredoxin reductase and a specific hydrogen donor for T4 ribonucleotide reductase that has no activity with *E. coli* host thioredoxin-(SH)₂ (1, 2). The primary structures of T4 and *E. coli* thioredoxin are not homologous, yet the tertiary structures determined by x-ray crystallography are similar (1). T4 thioredoxin and *E. coli* glutaredoxin are homologous proteins. T4 thioredoxin is functionally similar to glutaredoxin by being a GSH-disulfide-transhydrogenase and catalyzing GSH-dependent ribonucleotide reduction (31); in fact, the phage seems to have invented an ingenious way of obtaining reducing equivalents for its own ribonucleotide reductase from both the glutaredoxin and thioredoxin systems due to enzyme specificities and the difference in redox potentials (1, 31). A principal hydrogen donor for the herpes simplex virus type 1-encoded ribonucleotide reductase is a cellular thiore-

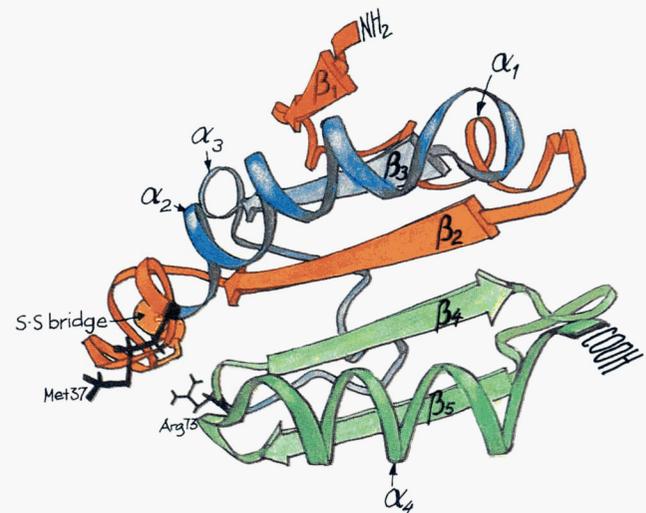


FIG. 2. Three-dimensional structure of *E. coli* thioredoxin-S₂ from x-ray crystallography at 2.8-Å resolution (1, 33). The color code (red, 1–37; blue, 38–73; and green, 74–108) refers to the fragments generated by selective cleavage at Met-37 or Arg-73.

doxin; no evidence for a major virus-coded thioredoxin was obtained (32).

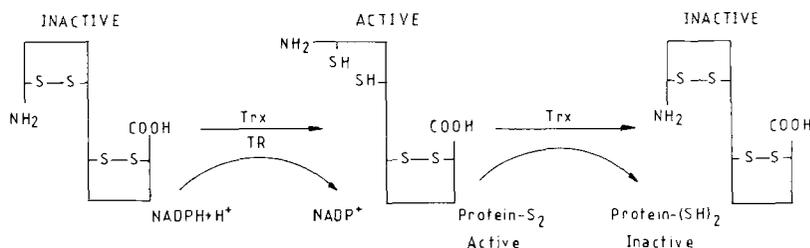
Structure of Thioredoxin and Glutaredoxin

Thioredoxin has been isolated and sequenced from a wide variety of prokaryotic and eukaryotic species (1, 7). *E. coli* thioredoxin is best characterized and contains 108 amino acid residues; thioredoxin-S₂ has a known three-dimensional structure to 2.8-Å resolution by x-ray crystallography (33) (Fig. 2). All species have at least one thioredoxin with an M_r around 12,000 and the same active site, Cys-Gly-Pro-Cys (1, 7, 34–37). Prokaryotic thioredoxins show about 50% sequence homology (7). Thioredoxins in mammalian cells (rabbit and calf²) are 90% similar and have 27% overall homology to the *E. coli* protein. The active site region is highly conserved with the consensus sequence: Val-Asp-Phe-Xaa-Ala-Xaa-Trp-Cys-Gly-Pro-Cys-(Lys)-(Met)-(Ile)-Xaa-Pro.

Glutaredoxin from *E. coli* contains 85 residues (38), and the calf thymus protein has 105 residues (39, 40). Both contain the same

² A. Holmgren, C. Palmberg, H. Jörnvall, and L. Hernberg, *J. Biol. Chem.*, submitted for publication.

FIG. 3. Outline of the regulation of protein activity by thiol redox control in the presence of the thioredoxin system. *Trx*, thioredoxin; *TR*, thioredoxin reductase.



active center: Cys-Pro-Tyr-Cys. These two glutaredoxins show about 30% identical residues and have essentially no sequence homology to the corresponding thioredoxins. Calf thymus glutaredoxin is highly similar to a GSH-disulfide transhydrogenase also called thioltransferase (39, 40).

Thioredoxin from *E. coli* is a compact almost spherical molecule with a central core of β -pleated sheet flanked on either side by helices (33) (Fig. 2). Residues 29–37 including the active site form a protrusion on the surface of the protein. The active site 14-membered disulfide ring is located at the C-terminal end of a β -strand (β_2) and in the first turn of a long bent α -helix (α_2). The folding of T4 and *E. coli* thioredoxin-S₂ is similar despite the fact that their primary structures are different (1, 41). Since *E. coli* glutaredoxin is homologous to phage T4 thioredoxin, a model of glutaredoxin has been built on a vector display based on the known tertiary structure of T4 thioredoxin (41). By comparison of the model with the thioredoxin structures, a common flat and hydrophobic surface area on one side of the redox-active S–S bridge was identified. This area containing residues 33–34, 75–76, and 91–93 in *E. coli* thioredoxin is suggested to be involved in binding interactions with other proteins.

How are the structures of oxidized and reduced thioredoxin related? This is a key question to understand the enzymatic function of thioredoxin. A conformational change takes place upon reduction of thioredoxin-S₂, as shown by spectroscopic results (1). However, no x-ray crystallographic studies of thioredoxin-(SH)₂ have been possible yet. Recently, two-dimensional NMR techniques have been used to study the structure of thioredoxin from *E. coli*³ (42, 43). Complete sequence-specific resonance assignments for both oxidized and reduced thioredoxin have been obtained (43), and structures are being calculated for the reduced form with distance geometry. From a preliminary comparison of the resonance assignments of oxidized and reduced thioredoxin (44) only limited conformational changes are indicated. Major chemical shifts are found for a few residues in the β -strand immediately preceding the active site S–S bridge (β_2) (Fig. 2) and the active site itself. Additional resonance shifts are observed for several residues distant in the primary structure. These residues (including the unique *cis*-proline 76) form the flat hydrophobic surface close to the active site S–S bridge, which as described above was identified to be common in thioredoxins and the model of glutaredoxin (44); they are also conserved during evolution of thioredoxin (7).

Thioredoxin can be selectively cleaved into two sets of fragments that reconstitute noncovalently into native-like molecules (thioredoxin C' and thioredoxin T') with partial activity (see Fig. 2) (1). Studies of these and the conformational change in oxidoreduction of thioredoxin should provide results of interest for understanding protein folding in general.

Regulatory Roles of Thioredoxin

The thioredoxin system is a general disulfide reductase and catalyzes NADPH-dependent reductions of exposed S–S bridges in a variety of proteins (1, 45). Often selective reduction of specific bonds can be achieved and followed by a simple spectrophotometric technique by recording the oxidation of NADPH (45). Studies of insulin disulfide reduction have given information regarding the rates of individual reactions. Thioredoxin catalyzes reduction of insulin by dithiothreitol. Compared with dithiothreitol, thioredoxin-(SH)₂ reacts almost 10⁵ times faster at pH 7 as a reductant of insulin; thioredoxin-S₂ is reduced about 10³ times faster than insulin by dithiothreitol (45).

In plants thioredoxin is known to regulate photosynthetic enzymes

in the chloroplast by light (46). Electrons flow from light through chlorophyll and ferredoxin to the enzyme ferredoxin-thioredoxin reductase that will reduce several specific thioredoxins. These will then reductively activate specific enzymes like fructose 1,6-bisphosphatase of the Calvin cycle (46). In the dark, the enzymes are oxidized and deactivated. Recently, it was shown that thioredoxin is essential for photosynthetic growth of *Anacystis nidulans* (47). Thus, although thioredoxin is dispensable in *E. coli*, it is essential for photosynthetic growth.

Has thioredoxin a general role in enzyme regulation? Regulation of enzymes or receptors via reversible oxidation of dithiols to disulfides or thiol redox control (1) could influence many physiologically important reactions (Fig. 3), although information is yet limited. A good example in mammalian cells is the role of thioredoxin to activate the glucocorticoid receptor to a steroid binding state (48). Compared with regulations via phosphorylation, thiol redox control has been more difficult to study due to the high rates of thiol-disulfide interchange reactions. Newer techniques of site-directed mutagenesis will be useful.

Mammalian Thioredoxin and Thioredoxin Reductase

Thioredoxin and thioredoxin reductase from mammalian cells (49) show some important differences in comparison with the better known *E. coli* proteins. Thus, mammalian thioredoxin contains additional half-cystine residues apart from the active site. These structural residues easily undergo oxidation to disulfides accompanied by inactivation and aggregation of the protein² (49). Thioredoxin reductase from mammalian cells is also a dimeric enzyme but has a higher molecular weight than the *E. coli* and yeast enzymes (1, 49) with subunits of 58 kDa instead of 35 kDa. Of importance, the enzyme from mammalian cells also shows much broader specificity and reacts directly with a number of nondisulfide components (alloxan, menadiol, Cu²⁺ etc.) apart from thioredoxins from many species (1, 49).

Immunohistochemical investigations in mammalian cells have revealed that thioredoxin and thioredoxin reductase are widespread in different tissues and not related to growth and DNA synthesis (50). The finding of high levels of thioredoxin and thioredoxin reductase in *e.g.* nerve cells and their axons (51) and the localization at intracellular membranes particularly in secretory cells will require further investigation (52) relating to physiological stimuli (52). A role in disulfide formation and secretion is implicated.

The thioredoxin active site has been found in thioredoxin-like domains in several proteins of higher molecular weight. Recently, it was shown that protein disulfide isomerase contains two presumed active-site sequences with strong homology to *E. coli* thioredoxin (53). Protein disulfide isomerase is implicated in the formation of native disulfide bonds in proteins (54) and is localized in the endoplasmic reticulum. Recently, protein disulfide isomerase was shown to be a substrate for both thioredoxin reductase and thioredoxin-(SH)₂ and thus a member of the thioredoxin system.³ More surprising has been the finding of thioredoxin domains in a phosphoinositide-specific phospholipase C (55). The implications of this in signal transduction and cell metabolism are yet entirely unknown.

Another unexpected role of a thioredoxin in the growth regulation of human T-cells has recently been discovered (56). A factor (ADF) from adult T-cell leukemia-derived cells (human T-lymphocyte virus I (HTLV-I) caused), which has interleukin 2 receptor-inducing and growth stimulatory activity, has been found to be highly similar if not identical to thioredoxin. Results indicate that a fraction of thioredoxin is secreted from cells and operates as a growth factor by an autocrine mechanism. How this is accomplished, by a specific receptor for thioredoxin or whether the thiol-disulfide redox activity of thioredoxin is involved, remains to be determined. Obviously, further studies relating thioredoxin's role in growth control (35) will be

³ J. Lundström and A. Holmgren, *J. Biol. Chem.*, submitted for publication.

awaited with great interest. Recently sodium selenite, which has growth stimulatory or inhibitory actions on cells, was shown to be a substrate for mammalian thioredoxin and thioredoxin reductase⁴ causing extensive NADPH oxidation by redox cycling with oxygen.

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⁴ S. Kumar and A. Holmgren, *J. Biol. Chem.*, submitted for publication.

⁵ The listing is incomplete due to space limitations but will provide access to original literature. I apologize to those whose work is only indirectly cited.