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Review Article

Molecular Views of Redox Regulation: Three-Dimensional Structures of Redox Regulatory Proteins and Protein Complexes

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ABSTRACT

The last decade has witnessed the explosion of research on redox-controlled cellular and biochemical processes. Whereas the vast majority of these studies have centered on clinical, genetic, and biochemical aspects of redox signaling and regulation inside and outside the cell, a significant number of nuclear magnetic resonance (NMR) and crystallographic studies have been undertaken to obtain an atomic-level understanding of the mechanisms of the redox regulation. This review highlights the recent progress of three-dimensional structure determination of key proteins and protein complexes involved in redox regulation. An increased list of such class of protein structures and their complexes with ligands will provide invaluable insight into the molecular basis of redox-regulatory processes and may be useful for the future development of therapeutic agents for redox-related diseases. Antiox. Redox Signal. 2, 827–840.

INTRODUCTION

ALTHOUGH IT HAS LONG BEEN KNOWN that the oxidation state of cysteine residues plays an important role in structure and function of many proteins, it has only been recently realized that, similar to phosphorylation/dephosphorylation of hydroxyl amino acids in proteins, the oxidation-reduction of critical cysteines is also involved in regulating a variety of biological signaling processes such as photosynthesis, gene transcription, translation, and apoptosis (Danon and Mayfield, 1994; Powis *et al.*, 1995; Sen, 1998; Aslund and Beckwith, 1999). The concept of redox signaling and regulation has emerged as a result of intensive research in the last decade on many redox-controlled biochemical and cellular processes. The driving force for the cellular redox signaling appears to be the reactive oxygen species (ROS). Although high levels of ROS are known to damage protein/nucleic acid structures, which ultimately lead to the oxidative stress, low levels of ROS produced during normal cellular metabolism are now believed to fine-tune the protein functions by modulating the oxidation state of some redox-sensitive cysteine residues even in the reducing intracellular environment (Finkel, 1998; Rhee, 1999). A large number of proteins containing such redox-sensitive cysteines have been identified (Sen, 1998). In general, modulation of these cysteine residues lead to inter- or intramolecular disulfide bonds and glutathionylation, both of which can either activate or inactivate proteins by inducing conformational change or altering

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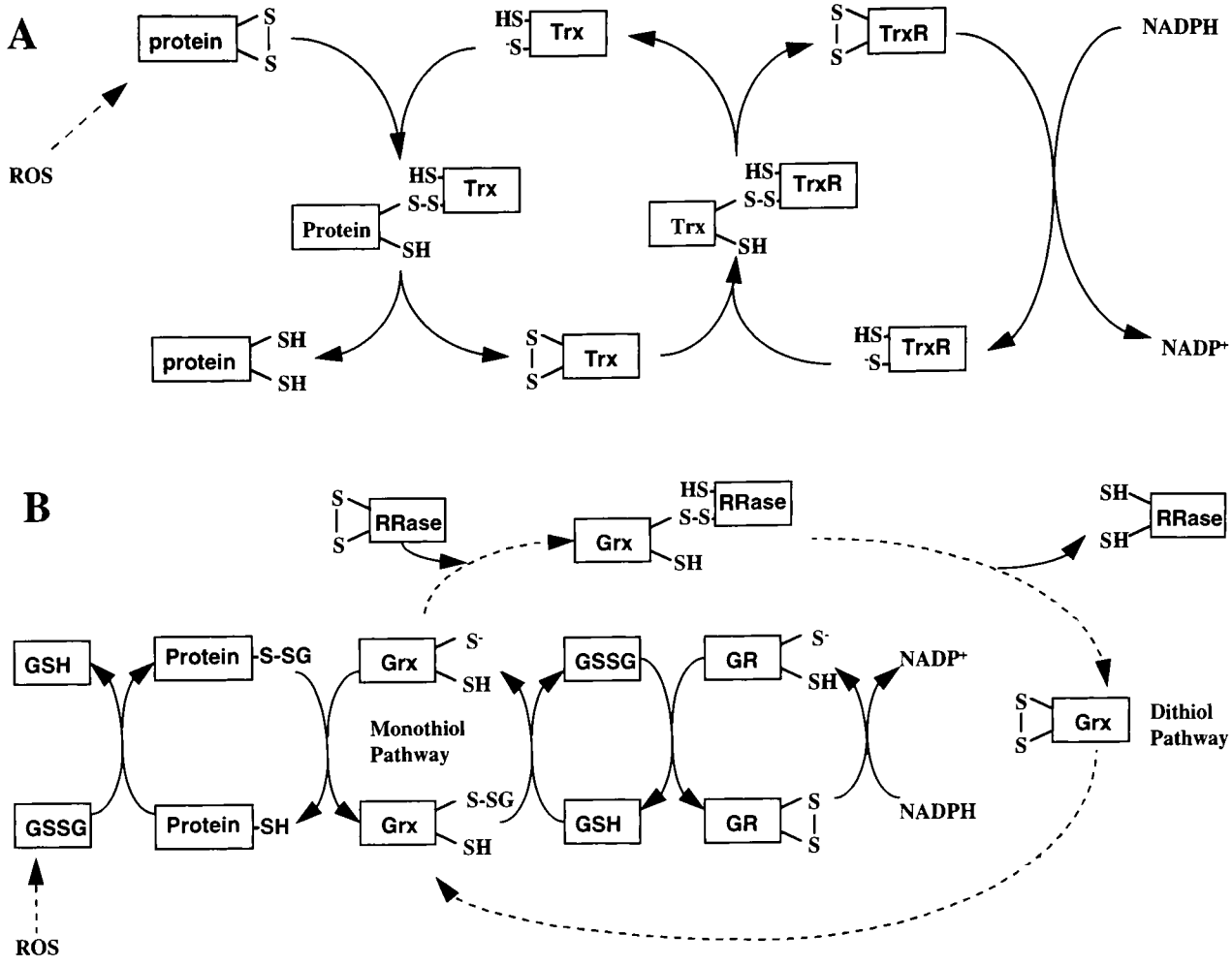

FIG. 1. Enzymatic reaction scheme for Trx and GSH-Grx systems. (**A**) Thioredoxin system. ROS-induced intra- or intermolecular disulfide bonds of the target proteins are reduced by the reduced Trx, which is regenerated by the TrxR coupled with NADPH. Note that for TrxRs from mammals and *C. elegans* containing additional redox center Cys-SeCys, the oxidized Trx can also catalyze the thioselenide formation for such pair, which regulates the TrxR activity (Lee *et al.*, 2000), reduction of the thioselenide is achieved by the redox center of TrxR adjacent to the FAD ring (Lee *et al.*, 2000). (**B**) GSH-Grx system. Two pathways are indicated: monothiol pathway catalyzing reduction of GSH-containing mixed disulfides and the dithiol pathway catalyzing reduction of ribonucleotide reductase (RRase) (dashed circle with arrows).

protein binding site. A structural elucidation of such modulation is exemplified by the pea fructose-1,6-bisphosphate phosphatase where a disulfide bridge promotes the disruption of the catalytic site across a distance of 20 Å and activation of this enzyme requires thioredoxin (Trx) (Chiadmi *et al.*, 1999). It has been suggested that the sensitive cysteine residues in some cases could also be oxidized to sulfenic (-Cys-SOH) or sulfinic acids (Cys-SO_2H) to regulate protein function, and such species have recently been observed in a number of proteins that are redox regulated (for review, see Claiborne *et al.*, 1999). Some proteins also contain selenocysteine residues that play a role in redox regulation. For example, in addition to having a redox center near the FAD ring, some thioredoxin reductases (TrxRs) from higher eukaryotes, such as mammals (Tamura and Stadtman, 1996; Zhong *et al.*, 2000) and the nematode worm *Caenorhabditis elegans* (Buettner *et al.*, 1999), contain an additional redox center comprised of Cys and selenocysteine (SeCys) residues in the order of -Gly-Cys-SeCys-Gly. Oxidation of SeCys leads to the formation of a thioselenide with the neighboring Cys (Lee *et al.*, 2000). Such oxidation or replacement of selenium with sulfur markedly reduces the TrxR activity, indicating an essential role for the SeCys in the redox regulation (Lee *et al.*, 2000).

There exist two major redox regulatory systems: the Trx system and the glutathione-glutaredoxin system. Both systems maintain the redox status of cysteine sulfhydryl groups through thiol-disulfide exchange reactions and both utilize NADPH as the cofactor for their respective regeneration systems. The reaction mechanisms, however, differ significantly (Fig. 1). A series of three-dimensional structures of proteins and protein complexes along the two reaction pathways have been determined, and significant insight into mechanisms in redox regulation has been obtained. The goal of this article is to provide an overview of the recent progress in structural studies of these two systems. Structures of other proteins involved in thiol-disulfide exchange, such as protein disulfide isomerases (PDI or DsbA) responsible for disulfide formation and protein folding (Kim and Mayfield, 1997), have also been solved and are summarized in Table 1 with the relevent Protein Data Base (PDB) codes, together with those from the Trx system and glutathione-glutaredoxin system.

TABLE 1. KNOWN STRUCTURES OF THIOL-DISULFIDE OXIDOREDUCTASES AND THEIR COMPLEXES[a]

Protein	*PDB Id*	*Year*[b]	*Organism*	*Mutation(s), redox state, etc.*	*Complex partner(s)*
Trx	1SRX	1976	*Escherichia coli*	oxidized	
Trx	2TRX	1991	*Escherichia coli*	oxidized	
Trx	3TRX	1992	*Homo sapiens*	reduced	
Trx	4TRX	1992	*Homo sapiens*	reduced	
Trx	2TIR	1993	*Escherichia coli*	K36E; oxidized	
Trx	1THO	1993	*Escherichia coli*	G33-R-P34 insertion; oxidized	
Trx	1TRS 1TRU	1994	*Homo sapiens*	C62A, C69A, C73A; oxidized	
Trx	1TRV 1TRW	1994	*Homo sapiens*	C62A, C69A, C73A; reduced	
Trx	1MDI 1MDJ 1MDK	1995	*Homo sapiens*	C35A, C62A, C69A, C73A	Mixed disulfide with NFκB peptide
Trx	1THX	1995	*Anabaena* sp.	oxidized	
Trx	1XOA	1996	*Escherichia coli*	oxidized	
Trx	1XOB	1996	*Escherichia coli*	reduced	
Trx	1CQG 1CQH	1996	*Homo sapiens*	C35A, C62A, C69A, C73A	Mixed disulfide with Ref-1 peptide
Trx	1ERT	1996	*Homo sapiens*	reduced	
Trx	1ERU	1996	*Homo sapiens*	oxidized	
Trx	1ERV	1996	*Homo sapiens*	C73S; reduced	
Trx	1ERW	1996	*Homo sapiens*	C32S, C35S	
Trx H	1TOF	1996	*Chlamydomonas reinhardtii*	oxidized	
Trx	1AIU	1997	*Homo sapiens*	D60N; reduced	
Trx	1AUC	1998	*Homo sapiens*	oxidized	
Trx	1T7P	1998	*Escherichia coli*	reduced	T7 DNA polymerase bound to DNA
Trx	1TXX	1999	*Escherichia coli*	P33V, G34W; oxidized	
Trx M	1DBY	1999	*Chlamydomonas reinhardtii*	oxidized	
Trx	1QUW	2000	*Bacillus acidocaldarius*	oxidized	
TrxR	1TDE	1994	*Escherichia coli*	oxidized	FAD
TrxR	1TDF	1994	*Escherichia coli*	C138S	FAD, $NADP^+$
TrxR	1TRB	1994	*Escherichia coli*	C138S	FAD
TrxR	1VDC	1997	*Arabidopsis thaliana*	oxidized	FAD
TrxR	1CL0	1999	*Escherichia coli*	reduced	FAD
FTR	1DJ7	2000	*Synethocystis* sp.		
Grx	1AAZ	1993	Bacteriophage T4	oxidized	
Grx	1ABA	1993	Bacteriophage T4	V15G, Y16P; oxidized	
Grx	1EGO	1993	*Escherichia coli*	oxidized	
Grx	1EGR	1993	*Escherichia coli*	reduced	
Grx	1GRX	1994	*Escherichia coli*	C14S	Mixed disulfide with glutathione

(continued)

TABLE 1. KNOWN STRUCTURES OF THIOL-DISULFIDE OXIDOREDUCTASES AND THEIR COMPLEXES[a] (CONT'D)

Protein	*PDB Id*	*Year*	*Organism*	*Mutation(s), redox state, etc.*	*Complex partner(s)*
Grx	1KTE	1996	*Sus scrofa*	oxidized	
Grx	1JHB	1998	*Homo sapiens*	reduced	
Grx	1DE1	1999	Bacteriophage T4	oxidized	
Grx	1DE2	1999	Bacteriophage T4	reduced	
Grx	1B4Q	1999	*Homo sapiens*	C7S, C25S, C78S, C82S	Mixed disulfide with glutathione
Grx 3	3GRX	1999	*Escherichia coli*	C14S, C65Y	Mixed disulfide with glutathione
Grx 1	1QFN	2000	*Escherichia coli*	C14S	Mixed disulfide with a peptide from ribonucleotide reductase
GR	3GRS	1998	*Homo sapiens*	oxidized	
GR	4GR1	1991	*Homo sapiens*	oxidized	Retro-GSSG[c]
GR	1GER	1994	*Escherichia coli*		FAD
GR	1GET	1994	*Escherichia coli*		$NADP^+$ and FAD
GR	1GES	1994	*Escherichia coli*	A179G, A183G, V197E, R198M, K199F, H200D, R204P	NAD^+
GR	1GEU	1994	*Escherichia coli*	A179G, A183G, V197E, R198M, K199F, H200D, R204P	NAD^+ and FAD
GR	1GRA	1994	*Homo sapiens*	oxidized	GSSG and $NADP^+$
GR	1GRB	1994	*Homo sapiens*	oxidized	NADH and PO_4^{3-}
GR	1GRE	1994	*Homo sapiens*		glutathione and PO_4^{3-}
GR	1GRF	1994	*Homo sapiens*	C58 carboxymethylated	PO_4^{3-}
GR	1GRG	1994	*Homo sapiens*	C58 modified by BCNU[d]	PO_4^{3-}
GR	1GRH	1994	*Homo sapiens*	C58 modified by HECNU[e]	PO_4^{3-}
GR	1XAN	1996	*Homo sapiens*		Xanthene inhibitor
GR	1GRT	1997	*Homo sapiens*	A34E, R37W	FAD
GR	2GRT	1997	*Homo sapiens*	A34E, R37W	FAD and GSSG
GR	3GRT	1997	*Homo sapiens*	A34E, R37W	FAD and GSSG
GR	4GRT	1997	*Homo sapiens*	A34E, R37W	FAD and GSSG
GR	5GRT	1997	*Homo sapiens*	A34E, R37W	FAD and oxidized glutathionylspermidine substrate
GR	1DNC	1998	*Homo sapiens*	sulfinic acid group in C63	Mixed disulfide with glutathione
GR	1GSN	1998	*Homo sapiens*	sulfenic acid group in C63, C234, C284, C423	Mixed disulfide with glutathione
GR	1BWC	1999	*Homo sapiens*		
DsbA[f]	1DSB	1994	*Escherichia coli*	oxidized	
DsbA	1FVK	1997	*Escherichia coli*	oxidized	
DsbA	1FVJ	1997	*Escherichia coli*	H32Y; oxidized	
DsbA	1AC1	1997	*Escherichia coli*	H32L; oxidized	
DsbA	1ACV	1997	*Escherichia coli*	H32S; reduced	
DsbA	1A23 1A24	1998	*Escherichia coli*	reduced	
DsbA	1A2L	1998	*Escherichia coli*	reduced	
DsbA	1A2M	1998	*Escherichia coli*	oxidized	
DsbA	1A2J	1998	*Escherichia coli*	oxidized	
DsbA	1BQ7	1999	*Escherichia coli*	P151A; oxidized	
TcpG	1BED	1997	*Vibrio cholerae*	oxidized	
PDO[g]	1A8L	1999	*Pyrococcus furiosus*	oxidized	
PDI	1MEK	1997	*Homo sapiens*	prolyl 4-hydroxylae β subunit; oxidized	
PDI	1BJX	1999	*Homo sapiens*	B domain	
PDI	2BJX	1999	*Homo sapiens*	B domain	
HAP1[h]	1BIX	1999	*Homo sapiens*		

[a]The list was compiled by searching PDB (www.rcsb.org/pdb/index.html) for respective protein names in compound information record and by text search. Horizontal lines separate different protein families.

[b]Year of the release of the coordinates by PDB (does not always coincide with publication year).

[c]N^4-(malonyl-*D*-cysteinyl)-L-2,4-diaminibutyrate disulfide.

[d]1,3-Bis (2-chloroethyl)-1-nitrosourea.

[e]1-(2-Chloroethyl)-3-(2-hydroxyethyl)-1-nitrosourea.

[f]*E. coli* DsbA, related TcpG from *V. cholerae*, eucaryotic PDI (protein disulfide isomerase) and homologous *P. furiosus* protein are all members of one family of protein disulfide oxidoreductases.

[g]Protein disulfide oxidoreductase.

[h]Synonym: Ref-1.

THIOREDOXIN SYSTEM

The enzymatic reaction mechanism of the Trx system is illustrated in Fig. 1A. Reduced Trx reduces the disulfide bonds of target proteins, becoming itself oxidized as a result. The regeneration of the reduced Trx is achieved by the enzyme TrxR and involves the transfer of electrons from NADPH to the active site disulfide bond of the TrxR, via FAD to the oxidized thioredoxin (Fig. 1A). Along the reaction pathway, several three-dimensional structures for proteins involved have been determined including TrxR (*E. coli* form), oxidized and reduced Trx (both human and *E. coli*), and human Trx complexed with two different protein target sites (NFκB and Ref-1). As discussed below, these structures have provided a framework for understanding how a key redox regulatory system, namely the Trx system, works.

Structure of TrxR

Structures of *E. coli* TrxR were solved by X-ray crystallography in three different forms: the oxidized form, a mutant form where its active site C138 was replaced by serine, and the C138S mutant complexed with $NADP^+$ (Kuriyan *et al.*, 1991; Waksman *et al.*, 1994). All three structures show essentially the same fold, with the proteins existing as dimers. Each monomer contains one FAD and one NADPH binding domain (Fig. 2A). The active site disulfide bond is located in the NADPH domain, stacked against the flavin ring in an orientation that allows reduction by the flavin. The nicotinamide ring, however, is quite distant from the flavin ring system (>17Å), and a large conformational change would be required to bring the two rings close enough for the hydride ion transfer (Fig. 2A). A 66° rotation of the NADPH domain about the two strands connecting the NADPH domain and the FAD domain needs to occur (Waksman *et al.*, 1994). Concomitantly, this rotation would allow the interaction of the substrate thioredoxin with the active site cysteine residues, that are otherwise inaccessible. Figure 3A displays a schematic model of such conformational change leading to the disulfide bond reduction. The crystal structure of reduced thioredoxin reductase without bound NADPH has also been recently reported (Lennon *et al.*, 1999) exhibiting no significant structural difference compared to the oxidized form. Interestingly, the flavin ring in the reduced form is bent compared to the planar conformation in the oxidized form, and the distortion is partly due to steric interactions between the ring and the reduced sulfur of Cys138, which may be important for catalysis. Crystal structure of an eukaryotic TrxR from the mustard plant *Arabidopsis thaliana* has also been solved at 2.5Å (Dai *et al.*, 1996). The relative positions of the domains in *A. thaliana* TrxR differ from those of the *E. coli* reductase. When the FAD domains are superimposed, the NADPH domain of *A. thaliana* TrxR must be rotated by 8° to superimpose on the corresponding domain of the *E. coli* enzyme. The domain rotation necessary for the thioredoxin reduction cycle is smaller in *A. thaliana* TrxR than that in *E. coli* reductase. A structure of TrxR complexed with both NADPH and thioredoxin would be necessary to obtain a complete picture of the enzymatic reaction, especially to visualize how the conformational change would occur upon binding to the substrate, Trx. Such work is being pursued (Wang *et al.*, 1996).

Very recently, the crystal structure of a functionally similar thioredoxin reductase in chloroplasts has been reported (Dai *et al.*, 2000). This enzyme ferredoxin:thioredoxin reductase (FTR) reduces disulfide bond of the oxidized plant Trx, thus mediating light-induced redox signaling. Different from *E. coli* TrxR, FTR requires ferredoxin for its disulfide bond reduction instead of NADPH, exhibits very different folding topology from TrxR, and is composed of an α-β heterodimer where the β subunit contains an iron–sulfur cluster and a redox active site. Remarkably, the Fe-S center and the redox active site sit on the opposite sides of the disk-like structure, which allows docking of a ferredoxin on one side for disulfide reduction of FTR and interaction with Trx on the other side for disulfide reduction of Trx. No major conformational change appears to be necessary for the FTR catalysis, in contrast to the *E. coli* TrxR.

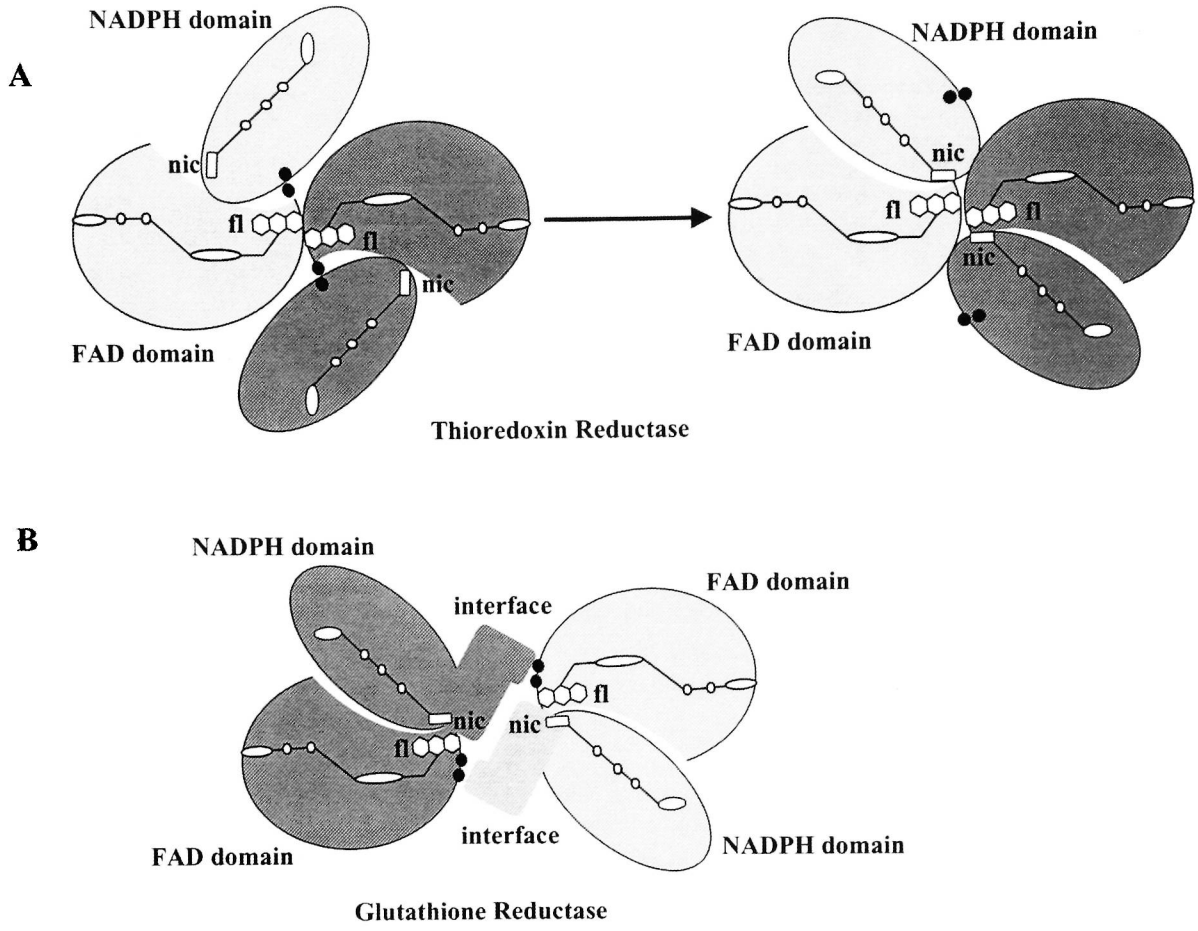

FIG. 3. Schematic representation of the dimeric forms of TrxR and GR (Waksman *et al.*, 1994). (**A**) TrxR. The model on the left depicts the structure of TrxR where nicotinamide and flavin rings are distant from each other and the active site disulfide bond is buried. A hypothetical active form of TrxR is illustrated on the right side, which requires a 66° rotation of NADPH domain with respect to the FAD domain. This would place the nicotinamide and flavin rings close enough for the electron transfer as well as expose the active site disulfide for interacting with the substrate, Trx. (**B**) GR. The nicotinamide ring, flavin ring, and the active site disulfide are adjacent to each other for the electron transfer. The flavin ring system and the nicotinamide ring are indicated by fl and nic. The disulfide bonds of TrxR and GR are indicated by two small black circles.

FIG. 2. Backbone structures of TrxR and GR monomers each containing a NADPH (green) and a FAD (pink) binding domain, respectively. (**A**) Monomer structure of *E. coli* TrxR (PDB code 1TDF) showing the positions of $NADP^+$, FAD, and active site cysteine residues. C138 was mutated to serine for crystallization purpose. Large conformational change is necessary for $NADP^+$-FAD electron transfer (see text and Fig. 3). (**B**) Monomer structure of GR (PDB code 1GET). In contrast to the large distance between $NADP^+$ and FAD in TrxR in (**A**), the $NADP^+$, FAD, and the active site disulfide in GR are adjacent to each other for ready electron transfer.

FIG. 4. Structures of Trx and Trx–target complexes. (**A**) Ribbon diagram of oxidized *E. coli* Trx (PDB code 2TRX) showing the central β-sheet surrounded by α-helices. (**B**) Backbone overlay of reduced (blue) (PDB code 1TRV) and oxidized (red) (PDB code 1TRS) human Trx showing the similarity with subtle differences around active site region. The active site cysteines in reduced Trx and the disulfide bond in oxidized Trx are colored in yellow. (**C**) Overlay of backbone structures of human Trx in the mixed disulfide complexes with NF-κB peptide (pink) (PDB code 1MDI) and Ref-1 (blue) (PDB code 1CQG), respectively, and *E. coli* Trx in the noncovalent complex with T7 DNA polymerase (green). Only the segment of T7 DNA polymerase covering the active site region of Trx is displayed. Notice the conserved binding site and extended structural feature shared by the three target molecules.

FIG. 5. Structures of Grx and Grx-SSG complexes. (**A**) the Ribbon diagram of oxidized pig liver Grx (PDB code 1KTE) showing the central β-sheet surrounded by α-helices, a similar fold as seen in Trx (see Fig. 4A). (**B**) Overlay of backbone structures of human Grx-SSG (1B4Q) and *E. coli* Grx-SSG (PDB code 1GRX) showing the similar binding mode.

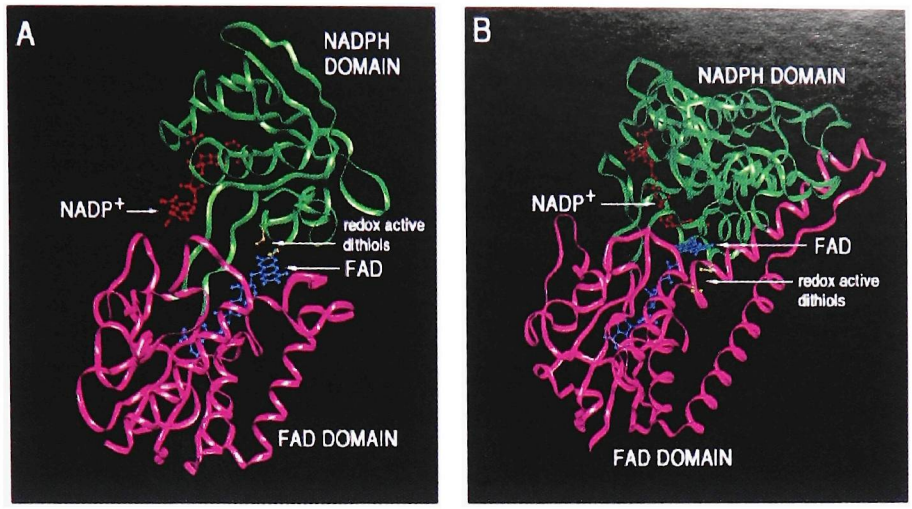

FIG. 2.

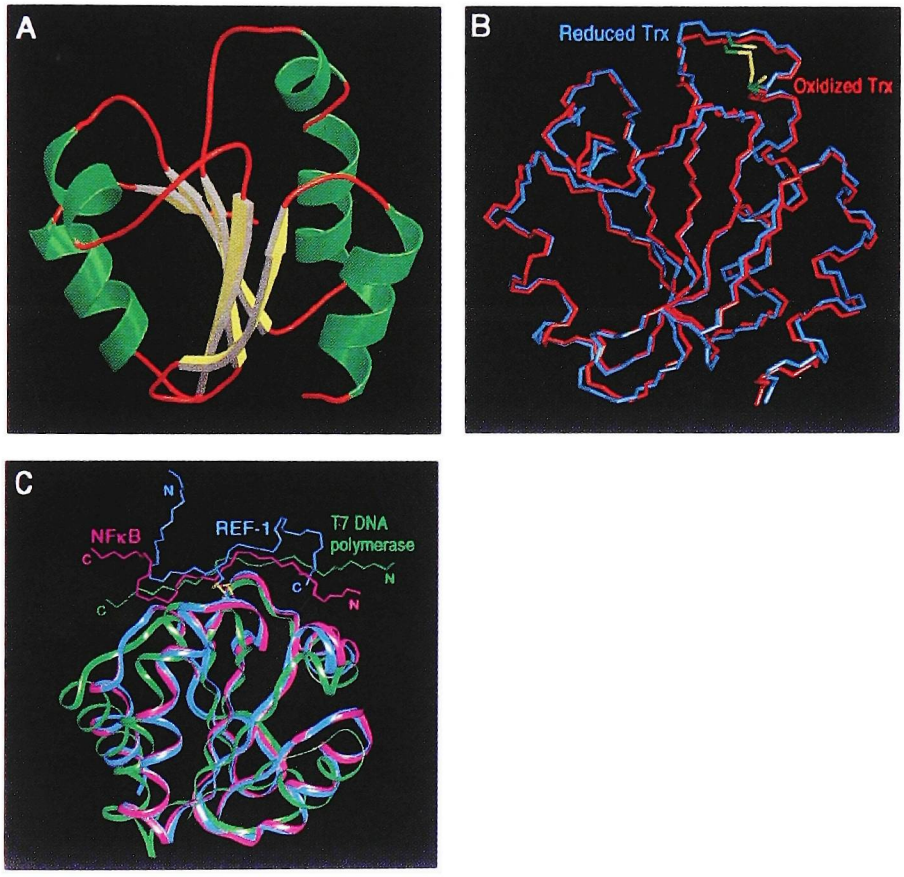

FIG. 4.

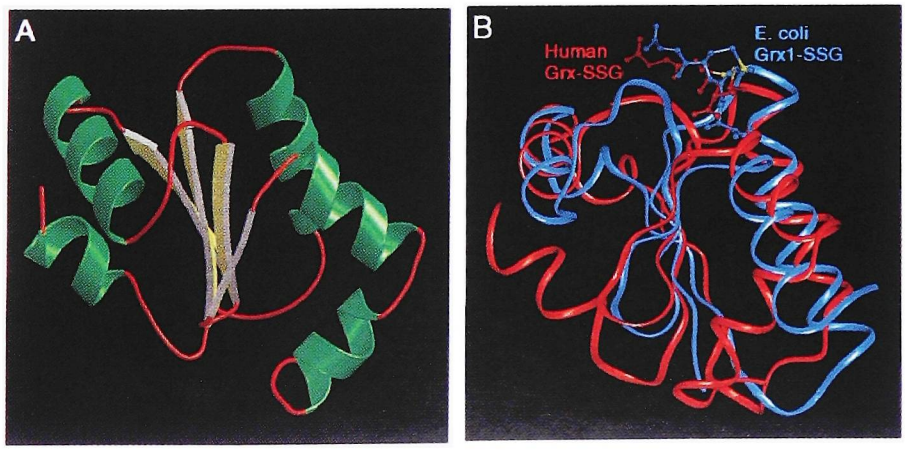

FIG. 5.

Structures of oxidized and reduced Trx

Once TrxR is reduced by NADPH, it targets the oxidized Trx to generate the reduced Trx (Fig. 1A). The oxidized Trx structure (*E. coli* form) was the first redox protein structure solved (Holmgren *et al.*, 1975) with 2.8 Å resolution and was later refined to 1.68 Å (Katti *et al.*, 1990). The structure has the fold of a central five β-stranded sheet surrounded by four α-helices and a dithiol/disulfide group in the active site, protruding from the protein surface (Fig. 4A,B). What had been puzzling was if there was any conformational difference between the oxidized and reduced forms for their different redox activities. Nuclear magnetic resonance (NMR) has played a major role in identifying the subtle structural (Fig. 4B) and dynamical differences. These differences were found to be localized around the active site between the two forms (Stone *et al.*, 1993; Qin *et al.*, 1994, 1996a; Jeng *et al.*, 1994, 1995), which likely play important role in the different redox activities of the two forms. Crystal structures of oxidized and reduced human Trx have also been recently reported (Weichsel *et al.*, 1996). Although the structures are largely similar to those obtained by NMR methods (Qin *et al.*, 1994), all of the crystal structures exist in inactive dimeric forms and dimer interface overlaps with the active site region. The physiological role of the dimer formation remains unknown. NMR relaxation measurements and analytical ultracentrifugation analysis have demonstrated that human Trx exists predominantly as a monomer in physiological condition (Gronenborn *et al.*, 1999). The crystal structure of oxidized *E. coli* Trx was also found in dimeric form (Holmgren *et al.*, 1975; Katti *et al.*, 1990) in contrast to the monomeric NMR structure (Jeng *et al.*, 1994). Therefore, it is possible that the dimer formation results from the crystal packing artifacts or differences in sample conditions (Gronenborn *et al.*, 1999).

Structures of Trx complexed with target molecules

Central to the redox regulatory function of the Trx system is the ability of the reduced Trx to recognize the target proteins for disulfide bond reduction. Because Trx can catalyze the disulfide bond reduction of a variety of proteins, it has been thought that the specificity of Trx is relatively low. This issue has been addressed by the NMR structure determination of human Trx complexed with two peptides derived from two different target proteins: the transcription factor NF-κB and Redox factor 1 (Ref-1), respectively (Qin *et al.*, 1995, 1996b).

Structure of human Trx in the mixed disufilde complex with NF-κB target peptide: NF-κB is a pivotal transcription factor that regulates expression of a wide variety of cellular and viral genes (Baeuerle and Baltimore, 1996; Baldwin, 1996). NF-κB exists as a heterodimer composed of a 50-kDa (p50) and 65-kDa (p65) subunit or as a p50 homodimer. The DNA binding activity of NF-κB has been shown to be redox regulated by Trx (Matthews *et al.*, 1992; Hayashi *et al.*, 1993; Hirota *et al.*, 1999). The oxidation of the Cys62 in p50 leads to the disulfide-linked dimer and inhibits the DNA binding of NF-κB, and the DNA binding can be restored in the presence of Trx (Matthews *et al.*, 1992; Hayashi *et al.*, 1993). The solution structure of a mixed disulfide-bonded complex between hTRX and a 13-residue peptide comprising residues 56–68 of the p50 subunit of NF-κB was determined. This portion of NF-κB, which is located in the L1 loop of p50, makes numerous contacts to DNA in the crystal structure of the NF-κB p50 homodimer bound to a κB site (Ghosh *et al.*, 1995; Muller *et al.*, 1995). The NF-κB peptide is located in a long boot-shaped cleft on the surface of human Trx delineated by the active-site loop, helices α2, α3, and α4, and strands β3 and β4 (Qin *et al.*, 1995). The peptide adopts a crescent-like conformation with a smooth 110° bend centered around residue 60 that permits it to follow the path of the cleft. In addition to the intermolecular disulfide bridge between Cys32 of Trx and Cys62 of the peptide, the complex is stabilized by numerous hydrogen-bonding, electrostatic, and hydrophobic interactions that involve residues 57–65 of the NF-κB peptide and hence confer substrate specificity.

Structure of Trx in the mixed disulfide complex with Ref-1 target peptide: The DNA-binding activity of the transcription factor AP-1 (a heterodimer of Fos and Jun) is redox-controlled by a protein called Ref-1/HAP1, which has both

DNA repair and redox activities (Abate *et al.*, 1990; Xanthoudakis *et al.*, 1992). The region essential for Ref-1 redox activity has been localized to the amino terminus of the protein, and site-directed mutagenesis has identified the cysteine at position 65 at the redox active site (Walker *et al.*, 1993; Xanthoudakis *et al.*, 1994). The exact molecular mechanism of how Ref-1 confers redox activity is not clear, even though the crystal structure of this protein is available (Gorman *et al.*, 1997). Nevertheless, upon oxidation, Ref-1 becomes redox inactive and is no longer able to activate AP-1. The activity of Ref-1 is restored by Trx (Xanthoudakis *et al.*, 1992). Indeed, a direct physical and functional interaction between Ref-1 and human Trx has been demonstrated both *in vivo* and *in vitro* using the yeast two-hybrid system and cross-linking (Hirota *et al.*, 1997). The three-dimensional structure of a complex of human Trx with a peptide comprising the relevant target site from Ref-1 (Qin *et al.*, 1996b) revealed that the Ref-1 peptide containing C65 is located, similar to the NF-κB target peptide, in a crescent-shaped groove on the surface of Trx. The groove is formed by residues in the active-site loop (residues 32–36), helix 3, β-strands 3 and 5, and the loop between β strands 3 and 4 (Qin et al., 1996b). However, the orientation of the Ref-1 peptide is opposite to that found in the complex of Trx with the NF-κB peptide (Fig. 4C). The orientations of the NF-κB and Ref-1 peptides appear to be determined by three discriminating interactions (Qin *et al.*, 1996b): the presence of an aromatic (or possible long hydrophobic chain) residue at P_{-2} or P_{+2} position that is buried in a deep hydrophobic pocket; the presence of an aliphatic residue at the P_{-4} or P_{+4} position to interact with the aromatic ring of Trp31 of hTRX; and the presence or absence of a positively charged residue at the P_{-5} or P_{+5} position, interacting with the side-chain carboxylates of Asp58 and Asp61 of hTRX.

The ability of Trx to recognize peptides in opposing orientations in the same binding groove indicates that this redox protein has succeeded in balancing versatility in substrate recognition with the requirements for access to substrates. Thereby, Trx has the potential to target a wide range of proteins with disulfides within the cell. The sequences around the disulfide-forming cysteines vary significantly. For example, peroxiredoxin, an antioxidant protein regulated by Trx, contains two conserved cysteines in the active site with one largely hydrophobic and the other partially exposed (Hirotsu *et al.*, 1999). The latter is likely to be the target site for Trx, but the sequence around the cysteine (DKHGEVCPAGWKP) is quite different from those from NF-κB (FRFRYVCEGPSHG) and Ref-1 (PATLKICSWNVDG). How Trx interacts with this region is not clear. More structures of Trx complexed with various targets are clearly necessary to deepen our understanding of the specificity of this ubiquitous enzyme.

Interestingly, in addition to its thiol-disulfide exchange activity, Trx is also known to regulate several important biochemical processes through protein–protein interactions, and such regulations is Trx oxidation-state-dependent (Russel and Model, 1986; Tabor *et al.*, 1987; Saitoh *et al.*, 1998; Nishiyama *et al.*, 1999). The structural basis of such regulation has been revealed by the recent crystal structure of reduced *E. Coli* Trx complexed with the target phage T7 DNA polymerase (Doublie *et al.*, 1998).

Structure of E. coli *Trx in a complex with phage T7 DNA polymerase:* During phage growth, the T7 DNA polymerase requires the tight binding of reduced *E. coli* Trx as its partner in 1:1 ratio ($K_d \sim$ n*M*) (Tabor *et al.*, 1987). This binding insures the high processivity of DNA replication; however, it was puzzling why the reduced Trx is required for this non-redox process since the structures of reduced and oxidized Trx are very similar (Fig. 4B) (Jeng *et al.*, 1994; Qin *et al.*, 1994). Crystal structure of reduced *E. coli* Trx complexed with T7 DNA polymerase shows that Trx binds to an extended loop (71 residues) located in the tip of a thumb of the processivity domain of the polymerase (Doublie *et al.*, 1998). This flexible binding loop, rich in glycine, proline, and lysine residues, wraps around the base of Trx and buries the active-site cysteines (Cys32 and Cys35) and the Arg73-Gly74-Ile-75-Pro76 segment. Although the structure of the reduced Trx in the complex is nearly identical to that of the oxidized form (Katti *et al.*, 1990), it is notable that C32 of the reduced Trx forms a hydrogen bond with

Thr327 of the loop, which likely contributes to the specificity of the reduced Trx in binding the polymerase. Moreover, it should be mentioned again that the subtle dynamical differences between the active site regions of the reduced and oxidized forms may also play an important role in fine-tuning the protein–protein recognition process (Stone *et al.*, 1993). Remarkably, Trx binding site with the polymerase overlaps with the same binding groove observed in those of Trx–NF-κB and Trx–Ref-1 complexes (Doublie *et al.*, 1998) (Fig. 4C). It will be interesting to see and compare how Trx interacts with other non-redox proteins such as ASK1 (Saitoh *et al.*, 1998) and TBP-2 (Nishiyama *et al.*, 1999) once these structures are available.

GLUTATHIONE-GLUTAREDOXIN SYSTEM

Figure 1B shows the schematic diagram of glutathione-glutaredoxin system. Key proteins involved in this reaction pathway include glutathione reductase (GR) and glutaredoxin (Grx). GrxR functions to reduce oxidized (GSSG) to reduced glutathione (GSH) during the reaction cycle. Although Grx is known to be able to mimic the catalytic mechanism of Trx in catalysis of ribonucleotide reductase-dependent formation of deoxyribonucleotides, which follows a dithiol reaction pathway (Fig. 1B) (Luthman and Holmgren, 1982; Bushweller *et al.*, 1992), it is generally highly selective for deglutathionylation of glutathione-containing mixed disulfides (*e.g.*, protein-SSG) via the monothiol reaction pathway (Fig. 1B) (Bushweller *et al.*, 1992; Gravina and Mieyal, 1993; Yang *et al.*, 1998). Because protein-SSG mixed disulfides are a prevalent form of cysteine modification in cells during oxidative stress (Chai *et al.*, 1994; Rokutan *et al.*, 1994; Rahman *et al.*, 1995; Ciriolo *et al.*, 1997; Cohen *et al.*, 1997), Grx likely plays a key catalytic role in redox-regulation of various cellular processes that involve glutathionylated proteins. In particular there are numerous examples of Grx catalysis of functional reactivation of glutathionylated proteins *in vitro*, such as phosphofructokinase (Yoshitake *et al.*, 1994), HIV-1 protease (Davis *et al.*, 1997), and the transcription factor NFI (Bandyopadhyay *et al.*, 1998).

Structure of GR

The structures of GR and its various complexes have been studied extensively, and its enzymatic mechanism is well-understood (Schulz *et al.*, 1978, 1982; Thieme *et al.*, 1981; Pai and Schulz, 1983; Karplus and Schulz, 1987, 1989; Pai *et al.*, 1988; Karplus *et al.*, 1989; Becker *et al.*, 1998). Similar to TrxR, GR is dimeric, with each monomer containing a FAD binding domain and NADPH binding domain (Fig. 2B). However, there are significant differences between the two forms: instead of more than 17 Å between the nicotinamide ring and flavin ring observed in the structure of TrxR, the two rings in Gr are very close to each other (Fig. 2B), which would not require a large conformational change for electron transfer as compared to TrxR. The enzyme's own disulfide bond, which is reduced by the flavin, is adjacent to the flavin ring system and sits at the base of a deep crevice in the structure formed at the dimer interface where glutathione binds. Figure 3B provides a schematic illustration of the Gr structure, indicating independent but nearby binding sites for FAD, NADPH, and GSSG as compared to those for TrxR in Fig. 3A.

Structure of oxidized and reduced Grx

As mentioned above, Grx acts as a dithiol to catalyze the reduction of either protein-SSG or ribonucleotide reductase. Structures of a number of Grxs from several species have been solved, including oxidized and reduced *E. coli* Grx1 Sodano *et al.*, 1991; Xia *et al.*, 1992), oxidized and reduced bacteriophage T4 Grx (Eklund *et al.*, 1992; Ingelman *et al.*, 1995), oxidized pig liver Grx (Katti *et al.*, 1995), and reduced human Grx (Sun *et al.*, 1998). All the structures have a similar thioredoxin fold (Fig. 5A). Comparison between oxidized and reduced *E. coli* Grx structures reveals very similar structures; however, differences were also found in local dynamics involving residues near the active site and the carboxy-terminal α-helix (Xia *et al.*, 1992; Kelley *et al.*, 1997). Moreover, the solvent-accessible surface area at the active site of reduced *E. coli* Grx is increased compared to the

oxidized form, which may be important for function (Xia *et al.*, 1992).

Structures of Grx complexed with target molecules

To understand the high specificity of Grx toward GSH-containing mixed disulfides, the structure of Grx complexed with GSH has been determined in three different forms: *E. coli* Grx1-SSG (Bushweller *et al.*, 1994), *E. coli* Grx3-SSG (Nordstrand *et al.*, 1999), and human Grx-SSG (Yang *et al.*, 1998). Except for some small differences, Grx in all of the three structures reveals a largely similar binding mode with GSH primarily via electrostatic interactions (Fig. 5B). On the other hand, the structure of *E. coli* Grx1 complexed with a target peptide derived from ribonucleotide reductase (737–761) containing the C754S mutation was recently reported (Berardi and Bushweller, 1999). The interactions between Grx1 and the peptide are also predominantly electrostatic. Although the binding induced significant conformational change in the helices of the protein, the peptide was shown to bind in a similar orientation as seen for the GSH mixed disulfide. However, a very different set of interactions was observed between the Grx–peptide and Grx–SSG complexes, suggesting that the active site of Grx has flexibility to adapt different substrates while achieving a high degree of specificity.

CONCLUSIONS

A significant number of structures involving proteins and protein complexes in the Trx system and GSH-Grx system have been determined (Table 1). These structures represent several key steps along the reaction pathways of the two systems and have provided a foundation for the current understanding of the enzymatic reactions involved in redox regulation. However, a full molecular picture of these two systems awaits for the completion of the structure determination of the complexes at all critical steps, such as TrxR complexed with Trx and Trx complexed with other target proteins. More biochemical/genetic experiments are necessary to demonstrate further the *in vitro* and *in vivo* significance of the redox regulatory processes via the redox-sensitive cysteines. These biochemical studies, combined with structural determination of inactive and active proteins containing the redox-sensitive cysteines, will provide more convincing and exciting molecular evidence of redox regulation occurring in diverse biological processes.

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ABBREVIATIONS

GR, Glutathione reductase; Grx, glutaredoxin; GSH, Glutathione; NMR, nuclear magnetic resonance; Trx, Thioredoxin; TrxR, thioredoxin reductase.

REFERENCES

ABATE, C., PATEL, L., RAUSCHER III, R.J., and CURRAN, T. (1990). Redox regulation of Fos and Jun DNA binding activity in vitro. Science **249,** 1157–1161.

ASLUND, F., and BECKWITH, J. (1999). Bridge over troubled waters: sensing stress by disulfide bond formation. Cell **96,** 751–753.

BANDYOPADHYAY, S., STARKE, D.W., MIEYAL, J.J., and GRONOSTAJSKI, R.M. (1998). Thioltransferase (glutaredoxin) reactivates the DNA-binding activity of oxidation-inactivated nuclear factor I. J. Biol. Chem. **273,** 392–397.

BAEUERLE, P.A., and BALTIMORE, D. (1996). NF-kappa B: ten years after. Cell **87,** 13–20.

BALDWIN, A.S., JR. (1996). The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu. Rev. Immunol. **14,** 649–683.

BECKER, K., SAVVIDES, S.N., KEESE, M., SCHIRMER, R.H., and KARPLUS, P.A. (1998). Enzyme inactivation through sulfhydryl oxidation by physiologic NO-carriers. Nature Struct. Biol. **5,** 267–271.

BERARDI, M.J., and BUSHWELLER, J.H. (1999). Binding specificity and mechanistic insight into glutaredoxin-catalyzed protein disulfide reduction. J. Mol. Biol. **292,** 151–161.

BUETTNER, C., HARNEY, J.W., and BERRY, M.J. (1999). The Caenorhabditis elegans homologue of thioredoxin reductase contains a selenocysteine insertion sequence (SECIS) element that differs from mammalian SECIS el-

ements but directs selenocysteine incorporation. J. Biol. Chem. **274,** 21598–21602.

BUSHWELLER, J.H., ASLUND, F., WÜTHRICH, K., and F., HOLMGREN, A. (1992). Structural and functional characterization of the mutant Escherichia coli glutaredoxin (C14——S) and its mixed disulfide with glutathione. Biochemistry **31,** 9288–9293.

BUSHWELLER, J.H., BILLETER, M., HOLMGREN, A., and WÜTHRICH, K. (1994). The nuclear magnetic resonance solution structure of the mixed disulfide between *Escherichia coli* glutaredoxin(C14S) and glutathione. J. Mol. Biol. **235,** 1585–1597.

CHAI, Y.C., HENDRICH, S., and THOMAS, J.A. (1994). Protein S-thiolation in hepatocytes stimulated by t-butyl hydroperoxide, menadione, and neutrophils. Arch. Biochem. Biophys. **310,** 264–272.

CHIADMI, M., NAVAZA, A., MIGINIAC-MASLOW, M., JACQUOT, J.P., and CHERFILS, J (1999). Redox signalling in the chloroplast: structure of oxidized pea fructose-1,6-bisphosphate phosphatase. EMBO J. **18,** 6809–6815.

CIRIOLO, M.R., PALAMARA, A.T., INCERPI, S., LAFAVIA, E., BUE, M.C., DE VITO, P., GARACI, E., and ROTILIO, G. (1997). Loss of GSH, oxidative stress, and decrease of intracellular pH as sequential steps in viral infection. J. Biol. Chem. **272,** 2700–2708.

CLAIBORNE, A., YEH, J.I., MALLETT, T.C., LUBA, J., CRANE, E.J., 3rd, CHARRIER, V., and PARSONAGE, D. (1999). Protein-sulfenic acids: diverse roles for an unlikely player in enzyme catalysis and redox regulation. Biochemistry **38,** 15407–15416.

COHEN, G., FAROOQUI, R., and KESLER, N. (1997). Parkinson disease: a new link between monoamine oxidase and mitochondrial electron flow. Proc. Natl. Acad. Sci. USA **94,** 4890–4894.

DAI, S., SAARINEN, M., RAMASWAMY, S., MEYER, Y., JACQUOT, J.P., and EKLUND, H. (1996). Crystal structure of Arabidopsis thaliana NADPH dependent thioredoxin reductase at 2.5 Å resolution. J Mol. Biol. **264,** 1044–1057.

DAI, S., SCHWENDTMAYER, C., SCHURMANN, P., RAMASWAMY, S., and EKLUND, H. (2000). Redox signaling in chloroplasts: cleavage of disulfides by an iron-sulfur cluster. Science **287,** 655–658.

DANON, A., and MAYFIELD, S.P. (1994). Light-regulated translation of chloroplast messenger RNAs through redox potential. Science **266,** 1717–1719.

DAVIS, D.A., NEWCOMB, F.M., STARKE, D.W., OTT, D.E., MIEYAL, J.J., and YARCHOAN, R. (1997). Thioltransferase (glutaredoxin) is detected within HIV-1 and can regulate the activity of glutathionylated HIV-1 protease in vitro. J. Biol. Chem. **272,** 25935–25940.

DOUBLIE, S., TABOR, S., LONG, A.M., RICHARDSON, C.C., and ELLENBERGER, T. (1998). Crystal structure of a bacteriophage T7 DNA replication complex at 2.2 A resolution. Nature **391,** 251–258.

EKLUND, H., INGELMAN, M., SODERBERG, B.O., UHLIN, T., NORDLUND, P., NIKKOLA, M., SONNERSTAM, U., JOELSON, T., and PETRATOS, K. (1992). Structure of oxidized bacteriophage T4 glutaredoxin (thioredoxin). Refinement of native and mutant proteins. J. Mol. Biol. **228,** 596–618.

FINKEL, T. (1998). Oxygen radicals and signaling. Curr. Opin. Cell. Biol. **10,** 248–253.

GHOSH, S., VAN DUYNE, G., GHOSH, S., and SIGLER, P. (1995). Structure of NF-κB p50 homodier bound to a κB site. Nature **373,** 303–310.

GORMAN, M., MORERA, S., ROTHWELL, D., DE LA FORTELLE, E., MOL, C., TAINER, J., HICKSON, I., and FREEMONT, P. (1997). The crystal structure of the human DNA repair endonuclease HAP1 suggests the recognition of extra-helical deoxyribose at DNA abasic sites. EMBO J. **16,** 6548–6558.

GRAVINA, S.A., and MIEYAL, J.J. (1993). Thioltransferase is a specific glutathionyl mixed disulfide oxidoreductase. Biochemistry **32,** 3368–3376.

GRONENBORN, A.M., CLORE, G.M., LOUIS, J.M., and WINGFIELD, P.T. (1999). Is human thioredoxin monomeric or dimeric? Protein Sci. **8,** 426–429.

HAYASHI, T., UENO, Y., and OKAMOTO, T. (1993). Oxidoredictive regulation of nuclear factor κB. J. Biol. Chem. **268,** 11380–11388.

HIROTA, K., MATSUI, M., IWATA, S., NISHIYAMA, A., MORI, K., and YODOI, J. (1997). AP-1 transcriptional activity is regulated by a direct association between thioredoxin and Ref-1. Proc. Natl. Acad. Sci. USA **94,** 3633–3638.

HIROTA, K., MURATA, M., SACHI, Y., NAKAMURA, H., TAKEUCHI, J., MORI, K., and YODOI, J. (1999). Distinct roles of thioredoxin in the cytoplasm and in the nucleus. A two-step mechanism of redox regulation of transcription factor NF-kappaB. J. Biol. Chem. **274,** 27891–27897.

HIROTSU, S., ABE, Y., OKADA, K., NAGAHARA, N., HORI, H., NISHINO, T., and HAKOSHIMA, T. (1999). Crystal structure of a multifunctional 2-Cys peroxiredoxin heme-binding protein 23 kDa/proliferation-associated gene product. Proc. Natl. Acad. Sci. USA **96,** 12333–12338.

HOLMGREN, A., SODERBERG, B.-O., EKLUNG, H., and BRANDEN, C.-I. (1975). Three dimensional structure of E. coli. thioredoxin-S2 to 2.8Å resolution. Proc. Natl. Acad. Sci. USA **72,** 2305–2309.

INGELMAN, M., NORDLUND, P., and EKLUND, H. (1995). The structure of a reduced mutant T4 glutaredoxin. FEBS Lett. **370,** 209–211.

JENG, M.F., and DYSON, H.J. (1995). Comparison of the hydrogen-exchange behavior of reduced and oxidized *Escherichia coli* thioredoxin. Biochemistry **34,** 611–619.

JENG, M.F., CAMPBELL, A.P., BEGLEY, T., HOLMGREN, A., CASE, D.A., WRIGHT, P.E., and DYSON, H.J. (1994). High-resolution solution structures of oxidized and reduced *Escherichia coli* thioredoxin. Structure **2,** 853–868.

KARPLUS, P.A., and SCHULTZ, G.E. (1987). Refined structure of glutathione reductase at 1.54Å resolution. J. Mol. Biol. **195,** 701–729.

KARPLUS, P.A., and SCHULTZ, G.E. (1989). Substrate binding and catalysis by glutathione reductase as de-

rived from refined enzyme: substrate crystal structures at 2Å resolution. J. Mol. Biol. **210,** 163–180.

KARPLUS, P.A., PAI, E.F., and SCHULTZ, G.E. (1989). A crystallographic study of the glutathione binding site of glutathione reductase at 0.3 nm resolution. Eur. J. Biochem. **178,** 693–703.

KATTI, S.K., LEMASTER, D.M., and EKLUND, H. (1990). Crystal structure of thioredoxin from *Escherichia coli* at 1.68 A resolution. J. Mol. Biol. **212,** 167–184.

KATTI, S.K., ROBBINS, A.H., YANG, Y., and WELLS, W.W. (1995). Crystal structure of thioltransferase at 2.2 A resolution. Protein Sci. **4,** 1998–2005.

KELLEY, J.J., 3rd, CAPUTO, T.M., EATON, S.F., LAUE, T.M., and BUSHWELLER, J.H. (1997). Comparison of backbone dynamics of reduced and oxidized *Escherichia coli* glutaredoxin-1 using ^{15}N NMR relaxation measurements. Biochemistry **36,** 5029–5044.

KIM, J., and MAYFIELD, S.P. (1997). Protein disulfide isomerase as a regulator of chloroplast translational activation. Science **278,** 1954–1957.

KURIYAN, J., KRISHNA, T.S., WONG, L., GUENTHER, B., PAHLER, A., WILLIAMS, C.H., JR., and MODEL, P. (1991). Convergent evolution of similar function in two structurally divergent enzymes. Nature **352,** 172–174.

LEE, S.R., BAR-NOY, S., KWON, J., LEVINE, R.L., STADTMAN, T.C., and RHEE, S.G. (2000). Mammalian thioredoxin reductase: oxidation of the C-terminal cysteine/selenocysteine active site forms a thioselenide, and replacement of selenium with sulfur markedly reduces catalytic activity. Proc. Natl. Acad. Sci. USA **97,** 2521–2526.

LENNON, B.W., WILLIAMS, C.H., JR., and LUDWIG, M.L. (1999). Crystal structure of reduced thioredoxin reductase from *Escherichia coli:* structural flexibility in the isoalloxazine ring of the flavin adenine dinucleotide cofactor. Protein Sci. **8,** 2366–2379.

LUTHMAN, M., and HOLMGREN, A. (1982). Rat liver thioredoxin and thioredoxin reductase: purification and characterization. J. Biol. Chem. **257,** 6686–6690.

MATTHEWS, J.R., WAKASUGI, N., VIRELIZIER, J.L., YODOI, J., and HAY, R.T. (1992). Thioredoxin regulates the DNA binding activity of NF-κB by reduction of a disulfide bond involving cysteine 62. Nucleic Acids Res. **20,** 3821–3830.

MULLER, C.W., REY, F.A., SODEOKA, M., VERDINE, G.L., and HARRISON, S.C. (1995). Structure of the NF-κB p50 homodimer bound to DNA. Nature **373,** 311–317.

NISHIYAMA, A., MATSUI, M., IWATA, S., HIROTA, K., MASUTANI, H., NAKAMURA, H., TAKAGI, Y., SONO, H., GON, Y., and YODOI, J. (1999). Identification of thioredoxin-binding protein-2/vitamin D(3) up-regulated protein 1 as a negative regulator of thioredoxin function and expression. J. Biol. Chem. **274,** 21645–21650.

NORDSTRAND, K., ASLUND, F., HOLMGREN, A., OTTING, G., and BERNDT, K.D. (1999). NMR structure of Escherichia coli glutaredoxin 3-glutathione mixed disulfide complex: implications for the enzymatic mechanism. J. Mol. Biol. **286,** 541–552.

PAI, E.F., and SCHULTZ, G.E. (1983). The catalytic mechanism of glutathione reductase as derived from X-ray diffraction analyses of reaction intermediates. J. Biol. Chem. **258,** 1752–1757.

PAI, E.F., KARPLUS, A.P., and SCHULTZ, G.E. (1988). Crystallographic analysis of the binding of NADPH, FAD to glutathione reductase. Biochemistry **27,** 4465–4474.

POWIS, G., BRIEHL, M., and OBLONG, J. (1995). Redox signaling and the control of cell growth and death. Pharmac. Ther. **68,** 149–173.

QIN, J., CLORE, G.M., and GRONENBORN, A.M. (1994). The high resolution three-dimensional structure solution structures of oxidized and reduced states of human thioredoxin. Structure **2,** 503–521.

QIN, J., CLORE, G.M., KENNEDY, W.M.P., HUTH, J., and GRONENBORN, A.M. (1995). Solution structure of human thioredoxin in a mixed disulfide intermediate complex with its target peptide from the transcription factor NFkB. Structure, **15,** 289–297.

QIN, J., CLORE, G.M., and GROENBORN, A.M. (1996a). Ionization equilibria for side-chain carboxyl groups in oxidized and reduced human thioredoxin and in the complex with its target peptide from the transcription factor NF kappa B. Biochemistry **35,** 7–13.

QIN, J., CLORE, G.M., KENNEDY, W.M.P., and GRONENBORN, A.M. (1996b). The solution structure of human thioredoxin complexed with its target from Ref-1 reveals peptide chain reversal. Structure **4,** 613–620.

RAHMAN, I., LI, X.Y., DONALDSON, K., HARRISON, D.J., and MACNEE, W. (1995). Glutathione homeostasis in alveolar epithelial cells in vitro and lung in vivo under oxidative stress. Am. J. Physiol. **269,** L285–L292.

RHEE, S.G. (1999). Redox signaling: hydrogen peroxide as intracellular messenger. Exp. Mol. Med. **31,** 53–59.

ROKUTAN, K., JOHNSTON, R.B., JR., and KAWAI, K. (1994). Oxidative stress induces S-thiolation of specific proteins in cultured gastric mucosal cells. Am. J. Physiol. **266,** G247–G254.

RUSSEL, M., and MODEL, P. (1986). The role of thioredoxin in filamentous phage assembly. Construction, isolation, and characterization of mutant thioredoxins. J. Biol. Chem. **261,** 14997–15005.

SAITOH, M., NISHITOH, H., FUJII, M., TAKEDA, K., TOBIUME, K., SAWADA, Y., KAWABATA, M., MIYAZONO, K., and ICHIJO, H. (1998). Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J. **17,** 2596–2606.

SCHULZ, G.E., SCHIRMER, R.H., SACHSENHEIMER, W., and PAI, E.E. (1978). The structure of the flavoenzyme glutathione reductase. Nature **273,** 120–124.

SCHULTZ, G.E., SCHIRMER, R.H., and PAI, E.F. (1982). FAD-binding site of glutathione reductase. J. Mol. Biol. **160,** 287–308.

SEN, C.K. (1998). Redox signaling and the emerging therapeutic potential of thiol antioxidants. Biochem. Pharmacol. **55,** 1747–1758.

SODANO, P., XIA, T.H., BUSHWELLER, J.H., BJORNBERG, O., HOLMGREN, A., BILLETER, M., and

WUTHRICH, K. (1991). Sequence-specific 1H n.m.r. assignments and determination of the three-dimensional structure of reduced *Escherichia coli* glutaredoxin. J. Mol. Biol. **221,** 1311–1324.

STONE, M.J., CHANDRASEKHAR, K., HOLMGREN, A., WRIGHT, P.E., and DYSON, H.J. (1993). Comparison of backbone and tryptophan side-chain dynamics of reduced and oxidized Escherichia coli thioredoxin using ^{15}N NMR relaxation measurements. Biochemistry **32:** 426–435.

SUN, C., BERARDI, M., and BUSHWELLER, J.H. (1998). The NMR structure of human glutaredoxin in the fully reduced form. J. Mol. Biol. **280,** 687–701.

TABOR, S., HUBER, H.E., and RICHARDSON, C.C. (1987). Escherichia coli thioredoxin confers processivity on the DNA polymerase activity of the gene 5 protein of bacteriophage T7. J. Biol. Chem. **262,** 16212–16223.

TAMURA, T., and STADTMAN, T.C. (1996). A new selenoprotein from human lung adenocarcinoma cells: purification, properties, and thioredoxin reductase activity. Proc. Natl. Acad. Sci. USA **93,** 1006–1011.

THIEME, R., PAI, E.F., SCHIRMER, R.H., and SCHULZ, G.E. (1981). Three dimensional structure of glutathione reductase at 2Å resolution. J. Mol. Biol. **152,** 763–782.

WAKSMAN, G., KRISHNA, T.S., WILLIAMS, C.H., JR., and KURIYAN, J. (1994). Crystal structure of Escherichia coli thioredoxin reductase refined at 2 Å resolution. Implications for a large conformational change during catalysis. J. Mol. Biol. **236,** 800–816.

WALKER, L., ROBSON, C.N., BLACK, E., GILLESPIE, D., and HICKSON, I. (1993). Identification of residues in the human DNA repair enzyme HAP1 (Ref-1) that are essential for redox regulation of Jun DNA binding. Mol. Cell. Biol. **13,** 5370–5376.

WANG, P.F., VEINE, D.M., AHN, S.H., WILLIAMS, C.H., JR. (1996). A stable mixed disulfide between thioredoxin reductase and its substrate, thioredoxin: preparation and characterization. Biochemistry **35,** 4812–4819.

WEICHSEL, A., GASDASKA, J.R., POWIS, G., and MONTFORT, W.R. (1996). Crystal structures of reduced, oxidized, and mutated human thioredoxins: evidence for a regulatory homodimer. Structure **4,** 735–751.

XANTHOUDAKIS, S., MIAO, G.G., WANG, F., E.PAN, Y., and CURRAN, T. (1992). Redox activation of Fos-Jun DNA binding activity is mediated by a DNA repair enzyme. EMBO J. **11,** 653–656.

XANTHOUDAKIS, S., MIAO, G.G., and CURRAN, T. (1994). The redox and DNA-repair activities of Ref-1 are encoded by nonoverlapping domains. Proc. Natl. Acad. Sci. USA **91,** 23–27.

XIA, T.H., BUSHWELLER, J.H., SODANO, P., BILLETER, M., BJORNBERG, O., HOLMGREN, A., and WUTHRICH, K. (1992). NMR structure of oxidized Escherichia coli glutaredoxin: comparison with reduced *E. coli* glutaredoxin and functionally related proteins. Protein Sci. **1,** 310–321.

YANG, Y., JIAO, S., NANDURI, R.S., STARKE, D.W., MIEYAL, J., and QIN, J. (1998). Reactivity of the human thioltransferase (C7S, C25S, C78S, C82S) mutant and NMR solution structure of its glutathionyl mixed-disulfide intermediate reflect catalytic specificity. Biochemistry **37,** 17145–17156.

YOSHITAKE, S., NANRI, H., FERNANDO, M.R., and MINAKAMI, S. (1994). Possible differences in the regenerative roles played by thioltransferase and thioredoxin for oxidatively damaged proteins. J. Biochem. **116,** 42–46.

ZHONG, L., ARNER, E.S., and HOLMGREN, A. (2000). Structure and mechanism of mammalian thioredoxin reductase: the active site is a redox-active selenol-thiol/selenenylsulfide formed from the conserved cysteine-selenocysteine sequence. Proc. Natl. Acad. Sci. USA **97,** 5854–5859.

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1. Benjamin Selles, Nicolas Rouhier, Kamel Chibani, Jeremy Couturier, Filipe Gama, Jean-Pierre JacquotChapter 13 Glutaredoxin **52**, 405-436. [CrossRef]
2. Jaanus Kruusma, Adam M. Benham, J. A. Gareth Williams, Ritu Kataky. 2006. An introduction to thiol redox proteins in the endoplasmic reticulum and a review of current electrochemical methods of detection of thiols. *The Analyst* **131**:4, 459. [CrossRef]
3. Nicolas Rouhier , Alexios Vlamis-Gardikas , Christopher Horst Lillig , Carsten Berndt , Jens-Dirk Schwenn , Arne Holmgren , Jean-Pierre Jacquot . 2003. Characterization of the Redox Properties of Poplar Glutaredoxin. *Antioxidants & Redox Signaling* **5**:1, 15-22. [Abstract] [Full Text PDF] [Full Text PDF with Links]
4. NICOLAS ROUHIER, ERIC GELHAYE, JEAN-PIERRE JACQUOT. 2002. Redox Control by Dithiol-Disulfide Exchange in Plants. *Annals of the New York Academy of Sciences* **973**:1, 520-528. [CrossRef]