Selenoproteins contain a highly reactive 21st amino acid selenocysteine (Sec) encoded by recoding of a predefined UGA codon. Because of a lack of selenoprotein supply, high chemical reactivity of Sec, and intricate translation machineries, selenoprotein crystal structures are difficult to obtain. Structural prerequisites for Sec involvement in enzyme catalysis are therefore sparsely known. Here we present the crystal structure of catalytically active rat thioredoxin reductase 1 (TrxR1), revealing surprises at the C-terminal Sec-containing active site in view of previous literature. The oxidized enzyme presents a selenenylsulfide motif in trans-configuration, with the selenium atom of Sec-498 positioned beneath the side chain of Tyr-116, thereby located far from the redox active moieties proposed to be involved in electron transport to the Sec-containing active site. Upon reduction to a selenolthiol motif, the Sec residue moved toward solvent exposure, consistent with its presumed role in reduction of TrxR1 substrates or as target of electrophilic agents inhibiting the enzyme. A Y116I mutation lowered catalytic efficiency in reduction of thioredoxin, but surprisingly increased turnover using 5-hydroxy-1,4-naphthoquinone (juglone) as substrate. The same mutation also decreased sensitivity to inhibition by cisplatin. The results suggest that Tyr-116 plays an important role for catalysis of TrxR1 by interacting with the selenenylsulfide of oxidized TrxR1, thereby facilitating its reduction in the reductive half-reaction of the enzyme. The interaction of a selenenylsulfide with the phenyl ring of a tyrosine, affecting turnover, switch of substrate specificity, and modulation of sensitivity to electrophilic agents, gives important clues into the mechanism of TrxR1, which is a selenoprotein that plays a major role for mammalian cell fate and function. The results also demonstrate that a recombinant selenoprotein TrxR can be produced in high amount and sufficient purity to enable crystal structure determination, which suggests that additional structural studies of these types of proteins are feasible.

Selenoproteins are a unique class of proteins found in many organisms from all domains of life (1–5). Several of the 25 selenoprotein-encoding genes identified in man are known to be important for human health and disease (6), and three mammalian selenoproteins have thus far been identified to be essential for embryonic development using mouse models. These selenoproteins are phospholipid glutathione peroxidase (7), cytosolic thioredoxin reductase (TrxR)2 (8), and mitochondrial thioredoxin reductase 2 (9). Selenoproteins contain the 21st amino acid selenocysteine (Sec), an analogue of cysteine (Cys), where selenium takes the place of sulfur. This sulfur-for-selenium substitution makes Sec a highly reactive nucleophile having a lower pK\textsubscript{a} value than Cys. The free selenolate of Sec can easily oxidize to form either selenenic or seleninic acid or, alternatively, a diselenide or selenenylsulfide with another Sec or Cys residue, respectively. Compounds, peptides, or proteins with diselenide or selenenylsulfide bonds typically display drastically lower redox potentials than the corresponding disulfide variants (3, 4, 10–12). The redox features and chemical properties of Sec are accordingly functionally utilized in selenoproteins, often being oxidoreductases with a single catalytic Sec residue in their active sites.

The structural basis for the use of Sec in selenoproteins is difficult to study because of limited availability of pure enzyme. Most selenoproteins are cumbersome to purify because of their low natural abundance and the high chemical reactivity of Sec that easily compromises the amino acid, and because of difficulties in production of synthetic selenoproteins. Barriers for straightforward recombinant production derive from the fact that Sec is co-translationally inserted at the position of a predefined UGA codon (normally a stop codon) by intricate translation machineries (13). Selenoprotein translation involves several special components, including a Sec-dedicated tRNA and a Sec-specific elongation factor. These interact with species- or domain-specific secondary structures in the selenoprotein mRNA, thereby identifying the UGA codon as Sec-encoding and not as a translational termination codon (10, 14–17). To our knowledge, the only native mammalian selenoprotein for which the crystal structure has yet been solved is that of glutathione peroxidase, purified from either bovine erythrocytes (18) or human plasma (19). Both these structures presented a
Sec residue oxidized to seleninic acid thereby representing an inactive hyperoxidized form of the enzyme, which may possibly be produced during catalysis as a dead-end oxidation product (18, 19). For mammalian selenoprotein enzymes found in nature, structure determinations illustrating the Sec position and structural context in an enzymatically active form of the protein have yet to be performed.

Mammalian thioredoxin reductases (TrxRs) are selenoproteins highly important for antioxidant defense, redox regulation of cell function, and support of cell proliferation (8, 9, 20–22). Mammalian TrxR was originally purified from bovine (23) or rat (24) tissues, with the human enzyme subsequently discovered to be a selenoprotein with its Sec residue located at the penultimate C-terminal position (25, 26). Soon it became clear that the C-terminal -Gly-Cys-Sec-Gly-COOH motif of the human selenoprotein was found in all mammalian TrxRs, and based upon the results of extensive enzymatic and biochemical analyses, the Cys-Sec dyad of this motif was shown to form a reversible selenenylsulfide/selenolthiol constituting the proper active site of the enzyme (27–31). Therefore, it is clearly different in structure and function from the smaller form of thioredoxin reductases found in lower organisms. In 2001, the first crystal structure of a Sec-to-Cys mutant of rat TrxR1 was published (32), followed by several structure determinations of similar Sec-substituted mutants, i.e. mutants of mouse TrxR2 (33–36), human TrxR1 (37) (also as directly deposited PDB entry 2CFY), and the non-selenoprotein orthologue of Drosophila (34, 35). These structures as well as additional modeling studies and several extensive biochemical analyses (27–43) have generated a generally accepted model for the major features of the catalytic mechanism of mammalian TrxRs. This model is summarized in Fig. 1. Shortly, it involves a head-to-tail arrangement of the two subunits in homodimeric FAD-containing TrxR1, displaying an overall structure and the first part of the reductive half-reaction closely similar to that of glutathione reductase. Fully oxidized enzyme (EHox, enzyme species 1 in Fig. 1) is first reduced by 1 eq of NADPH to the two-electron-reduced enzyme (EH2) involving reduced FADH2 (Fig. 1) is first reduced by 1 eq of NADPH to the two-electron-one reductase. Fully oxidized enzyme (EHox, enzyme species 1 in Fig. 1) is first reduced by 1 eq of NADPH to the two-electron-reduced enzyme (EH2) involving reduced FADH2 (species 2), making a charge-transfer complex with the second Cys in an N-terminal -CVNVGC- motif (species 3). The charge-transfer complex has a unique absorbance spectrum and is a feature shared with several enzymes of the pyridine nucleotide disulfide oxidoreductases, including glutathione reductase (27, 44). The corresponding motif of glutathione reductase is the proper active site of that enzyme, catalyzing reduction of glutathione disulfide. In mammalian TrxRs, however, this motif is instead believed to transfer electrons further to a C-terminal selenenylsulfide of the other subunit in the dimer, which thereby is considered to mimic the glutathione disulfide substrate of glutathione reductase, forming a selenenolthiol upon its reduction (species 4). The actual state of TrxR reducing its principal substrate thioredoxin is believed to be the four-electron reduced EH4 enzyme, made by reduction of EH3 with a second equivalent of NADPH and having a thiolate-FAD charge-transfer complex concomitant with a selenenolthiol at the C terminus (species 6). Upon reduction of thioredoxin, EH4 (species 3) is again formed. This mechanism postulates a reversible formation of the selenenylsulfide/selenolthiol motif at the C terminus

FIGURE 1. Scheme of mammalian TrxR catalysis. This scheme depicts one of two active sites in dimeric mammalian TrxR and the currently accepted model for its catalysis. The fully oxidized enzyme (Eox, species 1) is reduced by NADPH to the two-electron reduced enzyme (EH2), present in equilibrium between three principal forms (species 2–4). The charge-transfer complex found in species 3 is indicated by a dashed arrow. Upon reduction with a second equivalent of NADPH, four-electron reduced enzyme is formed (EH4, species 5 and 6). The EH4 enzyme with a C-terminal selenenolthiol motif present concomitant with a charge-transfer complex at the N-terminally located -CVNVGC- motif (species 6) is believed to be the form of the enzyme normally reducing its substrates with the selenenolthiol motif, thus forming a selenenylsulfide (species 6 transformed to species 3) upon catalysis (the oxidative half-reaction). Renewed formation of species 6 is possible after reduction of species 4 with another equivalent of NADPH (the reductive half-reaction) and catalysis is thus believed to normally occur with cycling of species 3–6. When the selenolate of the C terminus becomes exposed in the reduced enzyme, this can also be easily derivatized with electrophilic agents (X in the scheme), thus forming enzyme species irreversibly inhibited for thioredoxin reduction (red, species 7–9). In this study, cisplatin was used as electrophilic agent for analysis of the importance of Tyr-116 for this inhibitory reaction. The Sec-derivatized forms of the enzyme are completely inactive for Sec-dependent reduction of substrates such as thioredoxin, but NADPH can still reduce the FAD moiety, forming species 8 from species 7, and subsequently species 9 can be formed having a charge-transfer complex. The inhibited enzyme species 9 can promote Sec-independent reduction of some substrates with high efficiency, such as the quinone juglone analyzed in this study (Q in the scheme), or at about 5–10% efficiency compared with the native enzyme using DTNB as substrate (indicated by DTNB in parentheses in the scheme). The crystal structures determined in this study should correspond to species 1 and species 4 or species 6. For further details, see text.
Crystal Structure of the Selenoprotein TrxR1

and its direct interaction with the N-terminal thiolate-FAD charge-transfer complex. However, no structure has yet been determined for any Sec-containing TrxR that could confirm this assumption. The structure of the selenenylsulfide motif and its location in the enzyme has so far only been modeled. The prior structure determinations of Sec-to-Cys mutant forms of mammalian TrxR (32, 36, 37) furthermore revealed that the C-terminal tail seems to be highly flexible, with the configuration of the final C-terminal residues therefore difficult to model with any appreciable certainty.

Here we were able to determine the crystal structure of recombinantly produced Sec-containing rat TrxR1, and we could thereby assess a number of previous assumptions regarding the features of the C-terminal redox active motif. The results indeed confirm that a selenenylsulfide is formed at the C-terminal end of the oxidized enzyme. Unexpectedly, this motif was located far away from the corresponding site of the disulfide in oxidized glutathione in complex with glutathione reductase, where it has previously been modeled. Furthermore, the selenenylsulfide of TrxR1 presents a unique interaction with the phenyl ring of a tyrosine side chain in the active site (Tyr-116). Mutation of Tyr-116 resulted in lowered catalytic efficiency and thus confirmed its importance, but surprisingly, its mutation could also alter the substrate specificity of the enzyme, showing increased turnover with juglone as substrate with a Y116I (but not Y116T) mutation. The Tyr-116 mutation also conferred increased resistance to cisplatin, believed to target the Sec residue in the selenolthiol motif. Upon reduction of the C-terminal motif to a selenolthiol, the Sec side chain was indeed found relocated to a solvent-exposed position, consistent with its proposed major role in reduction of substrates for the enzyme. These results give insights into the structural basis for the function of Sec in TrxR1 and also raise questions regarding the mechanism of selenenylsulfide reduction during the reductive half-reaction of the enzyme.

EXPERIMENTAL PROCEDURES

Materials and Chemicals—If not specified, all chemicals were of high purity and were obtained from Sigma, and the sources for rare materials are mentioned in the text. Phenylarsine oxide-Sepharose was synthesized as described (45). TE buffer (50 mM Tris-HCl, 2 mM EDTA, pH 7.5) was used during the entire study if not stated otherwise.

Expression and Purification of TrxR1—Rat TrxR1 was expressed in Escherichia coli and purified over 2’,5’-ADP-Sepharose (GE Healthcare) as described previously, resulting in a mixture of selenium-containing and UGA-truncated enzyme (46). To get enriched selenium-containing enzyme, one more affinity chromatography using phenylarsine oxide-Sepharose was performed as described (45, 47). Shortly, the purified recombinant TrxR1 with about 15–25 units/mg specific activity in the standard DTNB assay (48) was reduced by 2 mM dithiothreitol for 20 min and then subjected to phenylarsine oxide-Sepharose pre-equilibrated with TE buffer. The column was then extensively washed with TE buffer containing 5 mM dithiothreitol to remove truncated protein. Subsequently enriched Sec-containing enzyme (specific activity at least 40 units/mg) was eluted using TE buffer with 10 mM sodium 2,3-dimercaptopropanesulfonate. The purity of the enzyme was confirmed by both SDS-PAGE and high pressure liquid chromatography using Superdex Gel Filtration Column 200 10/300 GL on an ÄKTAExplorer 10 system (GE Healthcare). The pure enzyme was subsequently desalted with NAP-25 column (GE Healthcare), concentrated over a 30-kDa cutoff ultracentrifuge device (PALL), and stored in TE buffer at 4 °C.

Construction of TrxR1 Tyrosine 116 Mutants—Two Tyr-116 TrxR1 mutants, with Tyr-116 changed to either isoleucine (Y116I) or threonine (Y116T), were produced. The cDNAs containing the corresponding mutations and the bacterial type SECIS element for Sec insertion (45, 49) were prepared using overlap extension PCR as described (50) (detailed PCR protocol and primer sequences are given in the supplemental material). After ligation of the fragments into the pET41a vector (Novagen) between the NdeI and EcoRI cloning sites, the mutations were confirmed by DNA sequencing (MWG Biotec). The plasmids were then transformed into E. coli BL21(DE3) followed by a co-transformation of the pSUABC plasmid (49), with expression and purification of full-length selenium-containing forms performed as for the wild type TrxR1 described above. We also attempted to make a Tyr-116 to phenylalanine variant but failed because of formation of nonsoluble protein and inclusion bodies that could not be recovered.

Crystallization—Purified recombinant rat TrxR1 with the same or slightly better enzymatic activity as found with purified native enzyme (at least 40 units/mg in the standard DTNB assay) was concentrated to 16 mg/ml in the presence of 10 mM NADP+. Crystals were grown by the vapor diffusion method, and the crystals used for data collections were obtained after 2–3 days in 15% polyethylene glycol 3350, 12% ethylene glycol, and 0.1 M HEPES, pH 7.5, in the hanging drop (1.5 μl protein + 1.5 μl reservoir).

Data Collection and Processing—Freezing of the crystals of TrxR1 did not require cryoprotection because of the presence of polyethylene glycol 3350 and ethylene glycol in the crystallization solution. The data were collected at tunable beamline 1911-3 (MAX Lab, Lund, Sweden). X-ray absorption spectroscopy showed a clear signal for selenium, and the data sets were collected at the peak of selenium absorption (λ = 0.978 Å).

Data sets from two different crystals of TrxR1 were obtained using different times for data collection. The 2.75-Å data set was collected with 25 s per image and Δφ = 0.8°, with 225 images used for the data set. The 3.1-Å data set was collected with 10 s and 1.2° per image. All diffraction data were processed with the program MOSFLM (51), scaled, and merged with SCALA from the CCP4 suite of programs (52). The data collection statistics are given in Table 1.

Structure Determination and Refinement—The cell dimensions of TrxR1 differed about 2 Å from the mutant protein (32), and we therefore used molecular replacement with the MOLREP program (53) to find the location of the protein in the cell. The estimation of the solvent content in the crystals of TrxR1 suggested that the asymmetric unit contained three dimers. A dimer of Sec-498 to Cys mutant TrxR1, PDB code 1h6v (32),
omitting the last five residues was used as a search model. The best solution had an \( R \) factor of 0.43. Ten cycles of rigid body refinement of the individual subunits using REFMAC5 and data in the 50.2–75.1 Å resolution interval (54) resulted in a decrease of \( R \) factor by 5%. Tight noncrystallographic symmetry restraints for all atoms were used in the refinement except for Trp-114, which obviously had different and multiple conformations in the subunits, and for the last six residues for which medium restraints were applied. Adjustments of the model was performed with COOT (55). Restrained refinement converged with \( R/\text{Rfree} \) 27.5/30.1%. TLS refinement with each subunit treated as a TLS group resulted in a large drop of the \( R \) factors (\( R/\text{Rfree} \) 20.6/23.6) without appreciable changes in the quality of the electron density map. The second data set, collected as quickly as possible to avoid reduction of the enzyme by intense x-ray radiation, gave usable data to only 3.1 Å and was refined as above but starting from the first model obtained. Refinements and model statistics for both data sets are summarized in Table 1.

Figs. 2–4 were made with PyMOL (56). The crystallographic data have been deposited with the Protein Data Bank, accession codes 3EA0 (TrxR1 with oxidized C terminus) and 3EAN (TrxR1 with reduced C terminus).

**Modeling**—A model of the complex with the mixed selenenylsulfide formed by Cys-32 of Trx and Sec-498 of TrxR1 was built in COOT (55), with the local geometry of the mixed selenenylsulfide bond based upon the geometry of the mixed disulfide bond found in the bacterial thioredoxin reductase-thioredoxin complex (PDB code 1FG6). A model of the oxidized selenenylcysteine motif moved toward the position of the disulfide of oxidized glutathione in its complex with glutathione reductase (PDB code 4GRT) was obtained by rotation of the C-terminal part of the chain around Gly-489, Gly-490, and Gly-496 and small adjustment of the chain. Idealization of the models was done in the end to ensure the absence of unfavorable contacts between atoms.

**Kinetics**—In the enzymatic assay of TrxR1 and its mutants using 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) as a model substrate (48), the formation of TNB was monitored as the decrease in absorbance at 340 nm (\( \lambda_{\text{abs}} \) 340 nm). In all other assays, the oxidation of NADPH was monitored as the increase in absorbance at 412 nm (\( \lambda_{\text{abs}} \) 412 nm = 13.6 mm \( \text{cm}^{-1} \)). The standard reaction mixture (50 μl) contained 0.2 mM NADPH and 3–15 nM enzyme in TE buffer with the reaction performed at 20 °C in a VersaMax spectrophotometer (Molecular Devices), using the same reaction mixture lacking enzyme as reference. For each data point, the initial velocity was determined in triplicate over at least five different substrate concentrations. Control assays, lacking the substrate, were routinely included as references. Kinetic constants were calculated with the Prism 5 software (GraphPad) after direct plotting of the catalytic velocity versus substrates concentration followed by automatic Michaelis-Menten fit with nonlinear regression.

**Inhibition by Cisplatin**—Enzyme preparations (1.6 μM TrxR1 or its mutants) were incubated in 85 μl of TE buffer with 0.3 mM NADPH for 5 min, followed by addition of 0.5 mM cisplatin (15 μl of Platinol® solution) for inhibition. At indicated time points, aliquots from the inhibition mixture were assayed in the standard DTNB assay (final concentration of 6.4 nM enzyme was used in the assay) (48). At the end of the experiment, an aliquot from the same mixture was also assayed in a Trx-coupled insulin reduction assay (48).

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**Table 1**

| Data collection | Oxidized | Reduced |
|----------------|----------|---------|
| Resolution     | 64.4–3.1 Å (3.27–3.1 Å) | 84.0–2.75 Å (2.9–2.75 Å) |
| Space group    | P2₁      | P2₁     |
| Cell dimensions | 78.2 Å, 137.7 Å, 168.9 Å, 94.0° | 78.6 Å, 140.7 Å, 171.2 Å, 94.5° |

**Refinement statistics**

|                         | Oxidized | Reduced |
|-------------------------|----------|---------|
| R<sub>free</sub>        | 25.9%    | 27.5%/20.6%* |
| R<sub>free</sub>        | 28.9%    | 30.1%/23.6%* |
| No. of atoms            | 23,102   | 23,204  |
| Protein                 | 22,556   | 22,556  |
| FAD                     | 318      | 318     |
| NADP*                   | 192      | 192     |
| Solvent                 | 36       | 138     |
| Total                   | 23,102   | 23,204  |
| Residues in disallowed regions | 0.0%  | 0.0%    |
| Residues in most favored regions | 86.0%* | 89.0%   |
| Bond angles             | 1.52     | 1.45    |
| Bond length             | 0.015    | 0.015   |
| Ramachandran plot       |          |         |

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* R and R<sub>free</sub> before and after TLS refinement.
* C-terminal residues are in a strained conformation with torsion angles in unfavorable regions of Ramachandran plot.
RESULTS

TrxR1 Crystallization, Data Collection, and Refinement—Numerous attempts to crystallize wild type TrxR1 in a wide range of crystallization conditions were performed, using more than 200 mg of TrxR1 having a specific activity of at least 40 units/mg, which is a slightly higher enzymatic activity than that of native TrxR1 purified from calf liver and thymus (23) or rat liver (24). Crystals obtained in different conditions were mainly rhomboid, but also hexagonal or spiked crystals were seen and usually gave very poor diffraction. Crystals that diffracted to reasonable resolution were finally obtained in the presence of 10 mM NADP+ from a solution of 15% PEG 3350, 12% ethylene glycol, and 0.1 M HEPES, pH 7.5. Although the Sec-containing wild type TrxR1 crystals were obtained at conditions different from Sec-498 to Cys mutant rat protein made previously (32), they belonged to the same space group P21 (32) and had similar, but not identical, cell dimensions (a = 78 Å, b = 138.3 Å, c = 170 Å, β = 94.5°).

Although the data were collected at the selenium peak wavelength, the anomalous contribution was negligibly low, which was not surprising for a 55-kDa protein with one selenium atom located in a rather flexible part of the chain. The strong x-ray radiation induced reduction of the C-terminal selenenylsulfide bridge, and therefore data sets with different x-ray exposure times were analyzed to obtain the structure of the oxidized enzyme. The R-factors of both the reduced and oxidized enzyme structures are very low for their resolution of the data due to the tightly restrained 6-fold symmetry and the use of TLS refinement.

Structure of TrxR1 and Its Sec-containing C-terminal Motif—The final models of TrxR1 showed good geometry and each consisted of 3 dimers, 2939 residues in total, 6 FAD molecules, and 6 NADP+ molecules (the nicotinamide-ribose moieties were not defined, as in the Sec-498 to Cys mutant protein structure (32)). The qualities of the electron density maps were very good, but the first 10–11 N-terminal residues were disordered in all subunits as were a number of surface residue side chains. The last six residues were shown to be very flexible with an ensemble of possible conformations, particularly in the reduced form of the enzyme. However, weak electron density for the C-terminal motif was observed for three subunits allowing definition of the general direction albeit not geometry of the chain and, most importantly, allowing the position and structure of the selenenylsulfide motif in the oxidized enzyme to be clearly determined (Fig. 2 and supplemental Fig. S1). The overall B-factor in the structure of the oxidized enzyme was about 56 Å2. This value increased gradually toward the C terminus from residue 485 and onward to reach a value of 95–96 Å2 for the last residues, including the selenenylsulfide motif, which was well defined in electron density (supplemental Fig. S1). In the reduced form the B-factor trend was the same, but the values were about 10 Å2 higher, and the electron density for the last residues was more ambiguous (supplemental Fig. S1).

The overall subunit structures were very similar to each other, also between the two data sets as well as compared with the Sec-498 to Cys mutant TrxR1 (32) (PDB code 1h6v) with root mean square deviation of 0.4–0.5 Å for main chain atoms of all residues in the chain. The C-terminal tail of the rat wild type TrxR1 was reduced to a selenolthiol in the 2.75 Å structure (Fig. 2A), although it was best modeled in the oxidized state in the 3.1 Å structure, with a covalent bond between selenium of Sec-498 and sulfur of Cys-497 (Fig. 2B). We believe that the reduction of the redox active motif of the C-terminal tail in the 2.75 Å structure was because of radiolysis. In that structure, the redox active disulfides in the CVNVC motif were also partly reduced, with average disulfide bonds significantly longer than ideal and some negative electron densities for the bonds in the $F_o - F_c$ difference map.

The C-terminal Sec-containing redox center in its oxidized state was located surprisingly far from N-terminal disulfide/dithiol motif, from which it is believed to receive reducing equivalents (Fig. 2B). In the oxidized state, the Sec involved in the selenenylsulfide bond was packed against the Tyr-116 phenol ring, and the oxidized selenenylsulfide motif was found in a peptide trans-configuration (Fig. 2B). In the reduced state, the sulfur of Cys-497 retained a position below the Tyr-116 ring, whereas Sec-498 with its selenium atom turned toward solvent and the presumed thioredoxin-binding site of the enzyme (Fig. 2A). Based on these structures, we modeled the TrxR1-Trx intermediary complex as a mixed selenenylsulfide between Cys-32 of Trx and Sec-498 of TrxR1, which could readily be built with only very minor shifts in the active site cleft upon regularization (Fig. 3, A and B). Several other models of TrxR1 were also built, where its C-terminal motif was positioned closer to the redox active motif in the N-terminal domain of the other subunit. One such model is shown in Fig. 3C, with the Cys-497-Sec-498 selenenylsulfide motif moved toward the position of oxidized glutathione in the crystal structure of the complex of GSSG with glutathione reductase, PDB code 2GRT (Fig. 3D). That model, requiring the least changes in conformation of the C-terminal residues, positioned Sec-498 significantly closer than Cys-497 to the N-terminal dithiol motif.

FIGURE 2. Structures of the Sec-containing C terminus with additional key residues and redox active moieties. Stereo view of the active site of reduced (A) and oxidized (B) TrxR1 is shown. One subunit is shown in green and another in yellow. Atom selenium in Sec-498 is shown in cyan. Cys-59 and Cys-64 are shown in the reduced form in reduced TrxR1, although they are only partly reduced in the structure, as discussed in the text.
with either a proposed Sec-498 to Cys-59 selenenylsulfide or a flexible nature of the C-terminal part, however, allows models of the oxidized C-terminal motif during the reduction of the C-terminal active site. The selenium atom was at least 2 Å closer to both the sulfur atom of Cys-32 of Trx and Sec-498 of TrxR1 (shown in detail in B), compared to His-472, where it has been positioned closer to the N-terminal redox active motif. D, comparison is shown between the locations of the selenenyl-sulfide of oxidized TrxR1 as found experimentally (see Fig. 2B), labeled “GSSG.” For further details and discussion, see the text.

The selenium atom was at least 2 Å closer to both the sulfur atom of Cys-59 (in the other subunit) and to His-472, compared with the sulfur atom of Cys-497, suggesting that Sec-498 should make a selenenylsulfide intermediate with the N-terminal motif during the reduction of the C-terminal active site. The flexible nature of the C-terminal part, however, allows models with either a proposed Sec-498 to Cys-59 selenenylsulfide or a Cys-497 to Cys-59 disulfide intermediate, as further discussed below (other models are not shown here).

Kinetics of Tyr-116 Mutants—To study the potential importance of Tyr-116 in TrxR1 for its catalysis, we constructed Y116F, Y116I, and Y116T mutants. The phenylalanine-substituted protein was not soluble and could thus not be analyzed further, whereas the other two mutants could be obtained in high yields and were purified as Sec-enriched proteins in the same manner as WT TrxR1. Both of these proteins were found to be catalytically active, but surprisingly, the substrate specificities of the two variants differed. Mammalian TrxR1 has wide substrate specificity, and therefore we first screened the enzyme activity of the mutant enzymes with several representative substrates, including DTNB (model disulfide substrate), selenite (non-disulfide selenium-containing substrate), juglone (quinone substrate), lipoamide (nonaromatic low molecular weight disulfide substrate), and thioredoxin (native principal substrate of TrxR1). For these different substrates, both Y116I and Y116T exhibited a lower catalytic efficiency compared with the wild type enzyme, but Y116I displayed a surprisingly elevated turnover utilizing juglone as substrate (Fig. 4). Steady-state kinetic constants of TrxR1 and the mutant proteins are summarized in Table 2. There was no major effect on $K_m$ for either DTNB or thioredoxin by the mutations, whereas $k_{cat}$ decreased to about half. For lipoamide, in contrast, the mutants showed a slightly higher $K_m$ and also higher $k_{cat}$ value compared with the wild type enzyme. Both mutants furthermore had increased $K_m$ and $k_{cat}$ values using juglone as substrate, and most surprisingly, Y116I showed about a 7-fold higher turnover with this substrate than with thioredoxin (Table 2). This suggested that Tyr-116 mutations may not only affect the catalytic efficacy of TrxR1 but also potentially divert electron flow to the advantage for some substrates, such as juglone, whereas other substrates, like Trx, became less efficiently reduced. Taking into account both $k_{cat}$ and $K_m$ values, however, the wild type enzyme showed better catalytic efficiency ($k_{cat}/K_m$) for all of the substrates (Table 2). Using the DTNB assay, we found no evident effect on the pH profile with any of the mutants, except for somewhat less reduction in turnover at increasingly alkaline conditions (supplemental Fig. S2).

Inhibition by Cisplatin—Asking whether Tyr-116 mutation could affect the susceptibility for targeting of the Sec residue with electrophilic compounds, we analyzed the inhibition pattern with cisplatin, a known inhibitor of the enzyme (57, 58). TrxR1 as well as both of the Tyr-116 mutants were inert to
cisplatin treatment under oxidized conditions, as expected because of a protection of the Sec residue when involved in a selenenylsulfide, although in agreement with earlier studies (57, 58) they became sensitive to inhibition by cisplatin upon reduction of the enzyme by NADPH (data not shown). Interestingly, however, the mutants, especially Y116I, retained considerable activity in the standard DTNB assay after 1 h of cisplatin treatment, whereas the wild type enzyme was fully inactivated under these conditions (Fig. 5A). All three enzymes, however, had been completely inactivated upon the cisplatin treatment when analyzed in the Trx-coupled insulin assay (Fig. 5B).

**DISCUSSION**

In this study, we have determined a structure of the unique selenenylsulfide-containing active site of the rat selenoprotein TrxR1. With this enzyme being important for mammalian cell function, redox control, and antioxidant defense (20, 22, 41) and a likely main target for anticancer therapy (21, 59, 60), it has evident medical value to characterize its active site and catalytic mechanism in molecular detail.

The most significant finding is the structural confirmation of the previously proposed selenenylsulfide bond between Cys-497 and Sec-498 in TrxR. Indeed, an easily accessible selenenylsulfide between two neighboring Cys and Sec residues is a highly unusual feature in proteins. Perhaps it is only found in nature in the Sec-containing thioredoxin reductases, because no other selenoproteins of mammals are known to contain neighboring Cys/Sec residues at their C-terminal end (61), and this feature apparently seems to be lacking among the more than 3600 selenoprotein genes recently found in the Global Ocean Sampling project (5). However, the human SepN selenoprotein implied in muscle disease (62) may have a similar structural motif within its internal SCUGS sequence (61), but the potentially redox active motif in SepN has not yet been functionally characterized. Early upon the discovery of mammalian TrxR as a selenoprotein (25, 26), it was shown that the Sec residue within its C-terminal -GCUG motif was essential for normal catalysis (28, 31). Already at that time it was also proposed that the Cys/Sec residues form a reversible selenolthiol/selenenylsulfide motif, as based upon kinetics analyses, peptide sequencing, and mass spectrometry (28, 30). It was furthermore proposed that the C terminus of the enzyme had to form some type of β-turn-like bend to position the sulfur and selenium atoms of the two side chains close to each other (30). Several studies have subsequently involved modeling of this motif and tried to position it into the active site of the enzyme (32, 33, 35–37), but its actual features in a crystal structure of the Sec-containing protein have not been determined until now. We are therefore able to analyze the features of this motif as found in the crystal structure, in view of the previous models and kinetic analyses, thereby probing the detailed molecular features in the catalytic cycle of rat TrxR1.

The packing of the selenenylsulfide motif close to the aromatic ring of the Tyr-116 side chain was at first peculiar to us. A corresponding Tyr residue is present in glutathione reductase and known to be important for catalysis in that enzyme (63), but in that case the hydroxyl group of the Tyr side chain is interacting with the disulfide of the oxidized glutathione substrate (63, 64). In contrast, in TrxR1 the planar surface of Tyr-116, not the

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**TABLE 2**

**Steady-state kinetic constants of wild type TrxR1 and its Tyr-116 mutants**

| Substrate | TrxR  | Y116I TrxR | Y116T TrxR |
|-----------|-------|------------|------------|
| DTNBb     | 4218 ± 187.5 | 2992 ± 171.5 | 2863 ± 143.8 |
| \( k_{cat} \) (min⁻¹) | 94.0 ± 11.9 | 97.1 ± 15.7 | 90.6 ± 13.2 |
| \( K_m \) (μM) | 44.9 | 30.8 | 31.6 |

**Jugloneb**

| Substrate | TrxR  | Y116I TrxR | Y116T TrxR |
|-----------|-------|------------|------------|
| \( k_{cat} \) (min⁻¹) | 1446 ± 56.0 | 10459 ± 635.1 | 3165 ± 183.3 |
| \( K_m \) (μM) | 2.27 ± 0.33 | 23.74 ± 2.83 | 6.98 ± 0.99 |
| \( k_{cat}/K_m \) (min⁻¹ μM⁻¹) | 637.0 | 440.6 | 453.4 |

**Human Trx**

| Substrate | TrxR  | Y116I TrxR | Y116T TrxR |
|-----------|-------|------------|------------|
| \( k_{cat} \) (min⁻¹) | 5650 ± 265.0 | 2459 ± 89.34 | 2691 ± 74.13 |
| \( K_m \) (μM) | 1.43 ± 0.19 | 0.99 ± 0.12 | 1.06 ± 0.093 |
| \( k_{cat}/K_m \) (min⁻¹ μM⁻¹) | 3956.6 | 2486.9 | 2529.1 |

**Lipoamide**

| Substrate | TrxR  | Y116I TrxR | Y116T TrxR |
|-----------|-------|------------|------------|
| \( k_{cat} \) (min⁻¹) | 1420 ± 89.6 | 1657 ± 103.5 | 1870 ± 207.0 |
| \( K_m \) (μM) | 2.89 ± 0.26 | 3.68 ± 0.31 | 5.59 ± 0.76 |
| \( k_{cat}/K_m \) (min⁻¹ μM⁻¹) | 492.0 | 450.9 | 334.8 |

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b \( k_{cat} \) per enzyme (dimer) and \( K_m \) were calculated following TNB formation at 412 nm.

b \( k_{cat} \) per enzyme (dimer) and \( K_m \) were calculated following NADPH oxidation at 340 nm.

Insulin-coupled Trx reduction assay is shown.

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**FIGURE 5. Inhibition by cisplatin.** TrxR1 and its Tyr-116 mutants were reduced by NADPH, followed by analysis of time-dependent inhibition of activity upon addition of cisplatin. A, activity in the standard DTNB assay is shown, analyzed at different time points with the activity before addition of cisplatin set to 100% for each enzyme preparation. B, the remaining activity after 60 min of incubation with cisplatin is shown using either DTNB or Trx as substrate. Please note the partial resistance to inhibition of DTNB, but not Trx, reduction in the mutants. wt, wild type.
Crystal Structure of the Selenoprotein TrxR1

FIGURE 6. Model of TrxR1 catalysis and the effects of Tyr-116. The prerequisites for this model postulates the following: Cys-64 makes the FAD-charge-transfer complex; Cys-59 reduces the selenenylsulfide by an intermediary selenenylsulfide interchange with Sec-498 with His-472 and Glu-477 potentially acting as proton acceptors (in two successive steps, through His-472); Trx becomes reduced through an intermediate selenenylsulfide between Cys-32 of Trx and Sec-498 of TrxR, and Tyr-116 acting by (i) facilitating selenenylsulfide reduction in the reductive half-reaction through resonance effects and, possibly, (ii) facilitating release of the intermediate selenenylsulfide with Trx by its interaction with the selenenylsulfide between Cys-497 and Sec-498. A, details of the reductive half-reaction. This model is in agreement with data in the literature and with modeling based upon the crystal structure, postulating that an intermediate selenenylsulfide between Cys-59 and Sec-498 is formed. Note, however, that formation of an intermediate disulfide between Cys-59 and Cys-497 cannot be excluded as an alternative pathway for the reductive half-reaction. B, details of a model for Trx reduction. This model postulates that Trx makes a mixed selenenylsulfide between Cys-32 and Sec-498, whereupon a thiolate is formed at Cys-497 (proton transferred to Cys-35 of Trx), which attacks the mixed selenenylsulfide, thereby enabling formation of the selenenylsulfide at the C terminus of TrxR1; this motif is subsequently interacting with Tyr-116. Numbering of all enzyme species in the figure is the same as in Fig. 1.

hydroxyl group, interacted with the selenenylsulfide of the C-terminal redox active motif. Moreover, the C-terminal selenenylsulfide as interacting with Tyr-116 of TrxR1 was found to be located further from the N-terminal FAD/dithiol(disulfide) motif than in the case of the disulfide in oxidized glutathione bound to glutathione reductase, or as modeled previously for TrxR1 (32–37). So how can Tyr-116 have such influence on the turnover in TrxR1, both in terms of catalytic efficiency, substrate preference, and susceptibility to inhibition by cisplatin, as found herein? Based upon the results of this study and considering prior literature, we suggest a detailed model for the catalytic mechanism of TrxR1, which subsequently can be scrutinized in forthcoming studies. In this model, we consider the following steps of the TrxR1 catalysis.

Reductive Half-reaction—The proposed detailed mechanism for the selenenylsulfide reduction within the reductive half-reaction of the enzyme is schematically depicted in Fig. 6A. One unresolved question in prior studies of mammalian TrxR1 is how the redox active site in the N-terminal domain of one subunit can reduce the selenenylsulfide at the C terminus of the other subunit. Based upon a number of studies with stopped-flow kinetics and other detailed biochemical analyses, it is generally accepted that NADPH first reduces the FAD, which subsequently forms a charge-transfer complex with one of the Cys residues of the CVNVGC motif, finally reducing the C-terminal motif (see Introduction and Fig. 1). It has not been clear, however, what intermediates are formed during this reduction and what residues are the leaving groups. The oxidized C terminus has previously been modeled with either its Sec (36) or Cys (32, 35) interacting with the N-terminal motif. The location of the selenenylsulfide motif as observed in the structure would not be compatible with a direct interaction with the FAD/dithiol motif. One possibility would therefore be a reduction of the selenenylsulfide through an electron relay through several additional residues from the N-terminal domain, even through a potential Tyr-116 tyrosyl radical intermediate, thereby participating in a long range selenenylsulfide reduction. However, analyses of the structure suggested no plausible electron path. We thus still find a direct interchange between the C-terminal disulfide and the N-terminal dithiol motifs to be the most probable event, which, however, necessitates that the selenenylsulfide motif moves from its location close to the Tyr-116 residue toward the N-terminal dithiol. Considering the evident mobility at the C terminus, as illustrated by the high B-factors and the earlier difficulties in detecting clear densities covering the C-terminal six residues (32, 36), we modeled the oxidized C terminus in a location closer to the N-terminal motif, keeping the features of the selenenylsulfide as found in the structure. In doing so, we found that Sec-498 was, for geometric reasons, clearly the most plausible residue to become positioned near the N-terminal redox active motif. We therefore suggest that it should be the most likely candidate to form a selenenylsulfide intermediate with Cys-59. However, an intermediate disulfide bond between Cys-59 and Cys-497 cannot be excluded even if that would require significantly larger movement and strain upon the structure. A similar (however not identical) location or modeling of the C-terminal tail of TrxR1 as in our model was proposed for the mitochondrial TrxR isoenzyme (36).
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(32) or human (37) Sec-498 to Cys mutant proteins, an intermediate between Cys-59 and Cys-497 was instead modeled for the reductive half-reaction, as also in other models (32, 35). The exact nature of the intersubunit redox exchange intermediate formed during the reductive half-reaction of the enzyme is thus clearly not yet known, but here we propose it to involve a selenenylsulfide between Sec-498 and Cys-59.

We further propose that the His-472 and Glu-477 dyads, argued by others to function together with Sec-498 as a “swapping catalytic triad” (12, 40, 65), serve the purpose of accepting the two protons needed for activation of the N-terminal FAD/dithiol motif into two thiolate anions, in two successive steps relayed through His-472. Such reactions would be reminiscent of the function of the corresponding His and Glu residues in glutathione reductase (64). We suggest that Tyr-116, perhaps through resonance effects, facilitates formation of the Cys-59/Sec-498 intermediate selenenylsulfide by weakening the C-terminal selenenylsulfide bond in its oxidized state. It is a well-known phenomenon that tyrosine residues can affect the formation and/or reactivity of dithiol/disulfide motifs, such as in the active sites of glutaredoxins (66) or during formation of folding intermediates (67). In other words, we propose that the interaction of the side chain of Tyr-116 with Sec in the selenenylsulfide of the oxidized C terminus may weaken this bond (increase its inherently low redox potential), so that its reduction by the N-terminal dithiol/charge-transfer complex becomes facilitated.

It should be noted that the structure of the C-terminal selenenylsulfide found to be in the peptide trans-configuration is in direct agreement with predictions that this configuration should be the most advantageous during catalysis involving the Sec-containing C-terminal motif (35). Finally, during the reductive half-reaction, we believe that the selenenylsulfide intermediate between Cys-59/Sec-498 should be relieved by attack of the Cys-64 thiolate, whereupon Cys-497 becomes protinated using a proton donated back from the His-472/Glu-477 couple and allowing it to pack with the aromatic ring of Tyr-116, as observed in the reduced structure. The Sec-498 residue, however, should stay mainly in its selenolate form because of the inherently low pKa of Sec, and it should readily move to a solvent-exposed position.

Oxidative Half-reaction—The proposed steps in the oxidative half-reaction are schematically depicted in Fig. 6B. The C-terminal selenenylsulfide of TrxR1 is reformed upon reduction of the active site disulfide between Cys-32 and Cys-35 in oxidized Trx, the principal substrate of TrxR1. For reduction of the disulfide of Trx, one of the two residues at the C-terminal selenolthiol of reduced TrxR should make an intermediate disulfide or selenenylsulfide with Trx. We propose that the Trx active site disulfide is attacked by the solvent-exposed selenolate of Sec-498, thereby forming an intermediary mixed selenenylsulfide with Cys-32 of Trx, as modeled in Fig. 4. Upon this event, we also propose that Cys-35 of Trx becomes protonated, whereas Cys-497 of TrxR is deprotonated forming a thiolate anion. This should occur either by a direct proton transfer from Cys-497 to Cys-35 of Trx or via solvent water molecules. The His-472/Glu-477 dyad is too far away to be active in these proton transfers. The intermediary selenenylsulfide between TrxR and Trx is then reduced by attack from the thiolate of Cys-497, releasing reduced Trx (with another proton uptake), and reforming the selenenylsulfide between Cys-497 and Sec-498 of TrxR. Possibly, Tyr-116 can facilitate also this step by interacting with the selenenylsulfide of the oxidized enzyme and thus lowering the threshold for Trx release, but this is less evident than the involvement of Tyr-116 in the reductive half-reaction.

Effects of the Tyr-116 Mutations—At first it was highly surprising to us that mutations of Tyr-116 could increase the kcat value for juglone by almost 10-fold (in the case of the Y116I substitution), while at the same time severely (but not completely) inhibiting the reduction of Trx. However, considering that juglone can be efficiently reduced by either the C-terminal selenolthiol motif or directly by the N-terminal FAD/dithiol motif of TrxR (38, 39, 68) and considering the plausible roles of Tyr-116 in the reductive and oxidative half-reactions, as described above, the effects of Tyr-116 mutations become explainable. Hence, we propose that the Tyr-116 substitutions diminish the possibility for the Cys-59 thiolate to attack Sec-498 of the selenenylsulfide, thereby mainly leaving the selenenylsulfide in the inactive oxidized state. The Cys-59 thiolate and charge-transfer-containing active site is thereby instead available for other types of reactions, including one- (or two-) electron reduction of quinones such as juglone. This line of reasoning is supported by the known high capacity of native, Sec-derivatized as well as Sec-lacking truncated forms of TrxR in reduction of juglone directly through the N-terminal redox active motif (38, 39, 68). Hence, without resonance effects of Tyr-116 and therefore a lack of an efficient reductive half-reaction, the result would easily be the observed increased propensity for juglone reduction but diminished activity with Trx as substrate. Similarly, the higher resistance to cisplatin derivatization upon Tyr-116 mutation also becomes understandable, because the selenolate of Sec-498 is the prime target for cisplatin targeting (57, 69), whereas the selenenylsulfide is essentially inert to reaction with electrophilic compounds (70). Again, if Tyr-116 mutations would lead to an attenuated reduction of the selenenylsulfide, according to the model put forward here, the observed effects on the kinetics of the Tyr-116 mutations become explicable.

Proposed Model for TrxR Catalysis in Relation to Prior Literature—It should be emphasized that the model for mammalian TrxR catalysis put forward here, albeit supported by the findings of this study and being in agreement with many prior studies, should be scrutinized in future studies and may prove to have to be revised. Even if we find this model to be the most plausible given our current knowledge of this enzyme, some caveats exist and deserve to be mentioned. The potential role of Tyr-116 must be further scrutinized. In the closely related glutathione reductase enzyme, as mentioned above, a homologous Tyr-116 residue interacts with the oxidized glutathione disulfide through the hydroxyl group (64). Also, in the mitochondrial TrxR2 isoenzymes of higher eukaryotes the corresponding residue is a His residue (36). Could these other enzymes involve their “Tyr-116 equivalents” for other types of effects than the selenenylsulfide-interacting resonance effects proposed here for rat TrxR1, or could a histidine fulfill the same role? The possibility that Tyr-116 in rat TrxR1 involves hydrogen bond-
ing to substrate, as with the corresponding Tyr residue in glutathione reductase, cannot be excluded, but considering that the mutant enzymes showed dual effects with a protection from inactivation by cisplatin and no increase in $K_m$ but rather decrease in $k_{cat}$ for Trx, we suggested that the role of Tyr-116 may be to facilitate the selenenylsulfide reduction step. Another issue to be addressed is that we propose the His-472/Glu-477 couple to act as proton acceptors/donors only in the reductive half-reaction, which is not compatible with the “Sec–498/His–472/Glu–477 swapping triad” as proposed by others on theoretical and thermodynamic modeling grounds (12, 40, 65). Furthermore, it was found earlier in the nonselenoprotein TrxR1 orthologue of the fruit fly, having a -Ser-Cys-Cys-Ser motif at the C terminus instead of a -Gly-Cys-Sec-Gly motif, that the flanking Ser residues in this motif could activate the Cys residues to approach the reactivity of the mammalian selenoprotein (41), whereas the mammalian enzyme cannot utilize the C-terminal motif derived from the fruit fly enzyme (43). Could a reason for these differences in effects of flanking Ser residues be found in the fact that with serines instead of glycines as flanking residues the structure of the oxidized C-terminal motif bound close to the Tyr-116 observed by us would be very strained and difficult to form? Finally, the exact redox exchange intermediate formed between the two subunits in the reductive half-reaction still needs to be conclusively identified. These and other questions should be addressed in future studies, but nonetheless we believe that the model for the rat TrxR1 catalysis as put forward here should be compatible with most, if not all, of the currently known features of the enzyme, including its kinetics and the features of the C-terminal motif as found in the crystal structure.

Concluding Remarks—Here we have described the structural characteristics of the unique selenenylsulfide/selenolthiol motif of rat TrxR1, and we put forward a detailed model for its involvement in the enzymatic catalysis, invoking a key role for the aromatic ring of Tyr-116. The mere fact that we were able to determine the crystal structure of a recombinantly expressed selenoprotein gives hope for a possibility to produce additional Sec-containing forms of TrxR (or potentially additional selenoproteins) in high enough amounts and purity for further detailed molecular and structural studies of these classes of proteins.

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REFERENCES

1. Flohe, L., Andreeisen, J. R., Brigelsius-Flohe, R., Maiorino, M., and Ursini, F. (2000) IUBMB Life 49, 411–420
2. Hatfield, D. L., and Gladyshev, V. N. (2002) Mol. Cell. Biol. 22, 3565–3576
3. Stadtman, T. C. (1996) Annu. Rev. Biochem. 65, 83–100
4. Böck, A., Forchhammer, K., Heider, J., Leinfelder, W., Sawers, G., Veprek, B., and Zinoni, F. (1991) Mol. Microbiol. 5, 515–520
5. Zhang, Y., and Gladyshev, V. N. (2008) PLoS Genet. 4, e1000095
6. Papp, L. V., Lu, J., Holmgren, A., and Khanna, K. K. (2007) Antioxid. Redox. Signal. 9, 775–806
7. Yant, L. J., Ran, Q., Rao, L., Van Remmen, H., Shibatani, T., Belter, J. G., Motta, L., Richardson, A., and Prolla, T. A. (2003) Free Radic. Biol. Med. 34, 496–502
8. Jakupoglu, C., Przemec, K. G., Schneider, M., Moreno, S. G., Mayr, N., Hatzopoulos, A. K., de Angelis, M. H., Wurst, W., Bornkamm, G. W., Brielmeier, M., and Conrad, M. (2005) Mol. Cell. Biol. 25, 1980–1988
9. Conrad, M., Jakupoglu, C., Moreno, S. G., Lipp, S., Banjac, A., Schneider, M., Beck, H., Hatzopoulos, A. K., Just, U., Sinowatz, F., Schmähl, W., Chien, K. R., Wurst, W., Bornkamm, G. W., and Brielmeier, M. (2004) Mol. Cell. Biol. 24, 9414–9423
10. Johansson, L., Gavelin, G., and Arné, E. S. J. (2005) Biochim. Biophys. Acta 1726, 1–13
11. Moroder, L. (2005) J. Pept. Sci. 11, 187–214
12. Wessjohann, L. A., Schneider, A., Abbas, M., and Brandt, W. (2007) Biochem. Biophys. Acta 1775, 576–582
13. Gronde-Culemann, E., Martin, G. W., III, Tujebajeva, R., Harney, J. W., and Berry, M. J. (2001) J. Mol. Biol. 310, 699–707
14. Drexel, C. M., and Copeland, P. R. (2003) Annu. Rev. Nutr. 23, 17–40
15. Hüttenhofer, A., and Böck, A. (1998) in RNA Structure and Function (Simons, R. W., and Grunberg-Manago, M., eds) pp. 603–639, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
16. Hatfield, D. L., Carlson, B. A., Xu, X. M., Mix, H., and Gladyshev, V. N. (2006) Prog. Nucleic Acids Res. Mol. Biol. 81, 97–142
17. Epp, O., Ladenstein, R., and Wendel, A. (1983) Eur. J. Biochem. 133, 51–69
18. Ren, B., Huang, W., Akesson, B., and Ladenstein, R. (1997) J. Mol. Biol. 268, 869–885
19. Arné, E. S. J., and Holmgren, A. (2000) Eur. J. Biochem. 267, 6102–6109
20. Gromer, S., Urig, S., and Becker, K. (2004) Med. Res. Rev. 24, 40–89
21. Lillig, C. H., and Holmgren, A. (2007) Antioxid. Redox. Signal. 9, 25–47
22. Holmgren, A. (1977) J. Biol. Chem. 252, 4600–4606
23. Holmgren, A., and Holmgren, A. (1982) Biochemistry 21, 6628–6633
24. Gladyshev, V. N., Jeang, K.-T., and Stadtman, T. C. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 6146–6151
25. Tamura, T., and Stadtman, T. C. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1006–1011
26. Arcsott, L. D., Gromer, S., Schirmer, R. H., and Becker, K. (2006) Mol. Cell. Biol. 26, 9472–9483
27. Eldred, J. R., Iwata, S., and Stadtman, T. C. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9533–9538
28. Arcsott, L. D., Gromer, S., Schirmer, R. H., and Becker, K. (2007) J. Mol. Biol. 370, 116–127
29. Arcsott, L. D., Gromer, S., Schirmer, R. H., and Becker, K. (2007) J. Mol. Biol. 370, 116–127
30. Arcsott, L. D., Gromer, S., Schirmer, R. H., and Becker, K. (2007) J. Mol. Biol. 370, 116–127
31. Arcsott, L. D., Gromer, S., Schirmer, R. H., and Becker, K. (2007) J. Mol. Biol. 370, 116–127
32. Arcsott, L. D., Gromer, S., Schirmer, R. H., and Becker, K. (2007) J. Mol. Biol. 370, 116–127
33. Arcsott, L. D., Gromer, S., Schirmer, R. H., and Becker, K. (2007) J. Mol. Biol. 370, 116–127
Crystal Structure of the Selenoprotein TrxR1

44. Williams, C. H., Jr. (1992) in Chemistry and Biochemistry of Flavoenzymes (Müller, F., ed) Vol. 3, pp. 121–211, CRC Press, Inc., Boca-Raton, FL
45. Cheng, Q., Stone-Elander, S., and Arnér, E. S. J. (2006) Nat. Protocols 1, 604–613
46. Rengby, O., Johansson, L., Carlson, L. A., Serini, E., Vlamis-Gardikas, A., Kårnsäs, P., and Arnér, E. S. J. (2004) Appl. Environ. Microbiol. 70, 5159–5167
47. Johansson, L., Chen, C., Thorell, J.-O., Fredriksson, A., Stone-Elander, S., Gafvelin, G., and Arnér, E. S. J. (2004) Nat. Meth. 1, 61–66
48. Arnér, E. S. J., and Holmgren, A. (2000) in Current Protocols in Toxicology (Maines, M., Costa, L., Reed, D., and Sassa, S., eds) pp. 7.4.1–7.4.14, John Wiley & Sons, Inc., New York
49. Arnér, E. S. J., Sarioglu, H., Lottspeich, F., Holmgren, A., and Böck, A. (1999) J. Mol. Biol. 292, 1003–1016
50. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K., and Pease, L. R. (1989) Gene (Amst.) 77, 51–59
51. Leslie, A. G. W. (1992) Joint CCP4 + ESF-EAMCB Newsletter on Protein Crystallography, No. 26
52. Collaborative Computational Project, Number 4 (1994) Acta Crystallogr. Sect. D Biol. Crystallogr. 50, 760–763
53. Vagin, A., and Teplyakov, A. (2000) Acta Crystallogr. Sect. D Biol. Crystallogr. 56, 1622–1624
54. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Acta Crystallogr. Sect. D Biol. Crystallogr. 53, 240–255
55. Emsley, P., and Cowtan, K. (2004) Acta Crystallogr. Sect. D Biol. Crystallogr. 60, 2126–2132
56. DeLano, W. L. (2002) The PyMOL Molecular Graphics System, DeLano Scientific, Palo Alto, CA
57. Witte, A. B., Anestá, K., Jerremalm, E., Ehsson, H., and Arnér, E. S. J. (2005) Free Radic. Biol. Med. 39, 696–703
58. Arnér, E. S. J., Nakamura, H., Sasada, T., Yodoi, J., Holmgren, A., and Spyrou, G. (2001) Free Radic. Biol. Med. 31, 1170–1178
59. Urig, S., and Becker, K. (2006) Semin. Cancer Biol. 16, 452–465
60. Arner, E. S., and Holmgren, A. (2006) Semin. Cancer Biol. 16, 420–426
61. Vagin, A., and Teplyakov, A. (2000) Acta Crystallogr. Sect. D Biol. Crystallogr. 56, 1622–1624
62. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Acta Crystallogr. Sect. D Biol. Crystallogr. 53, 240–255
63. Emsley, P., and Cowtan, K. (2004) Acta Crystallogr. Sect. D Biol. Crystallogr. 60, 2126–2132
64. DeLano, W. L. (2002) The PyMOL Molecular Graphics System, DeLano Scientific, Palo Alto, CA
65. Witte, A. B., Anestá, K., Jerremalm, E., Ehsson, H., and Arnér, E. S. J. (2005) Free Radic. Biol. Med. 39, 696–703
66. Arner, E. S., and Holmgren, A. (2006) Semin. Cancer Biol. 16, 420–426
67. Kryukov, G. V., Castellano, S., Novoselov, S. V., Lobanov, A. V., Zehtab, O., Guigo, R., and Gladyshev, V. N. (2003) Science 300, 1439–1443
68. Allamand, V., Richard, P., Lescreure, A., Ledeuil, C., Desjardin, D., Petit, N., Gartioux, C., Ferreiro, A., Krol, A., Pellegrini, N., Uturizbarea, J. A., and Guicheney, P. (2006) EMBO Rep. 7, 450–454
69. Krauth-Siegel, R. L., Arscott, I. D., Schönehen-Janas, A., Schirmer, R. H., and Williams, C. H., Jr. (1998) Biochemistry 37, 13968–13977
70. Pai, E. F., and Schultz, G. E. (1983) J. Biol. Chem. 258, 1752–1757
71. Brandt, W., and Wessjohann, L. A. (2005) ChemBioChem 6, 386–394
72. Holmgren, A. (2000) J. Mol. Biol. 303, 423–432
73. Zhang, J. X., and Goldenberg, D. P. (1997) Protein Sci. 6, 1549–1562
74. Anestá, K., Prast-Nielsen, S., Cenas, N., and Arnér, E. S. J. (2008) PLoS ONE 3, e1846
75. Anestá, K., and Arnér, E. S. J. (2003) J. Biol. Chem. 278, 15966–15972
76. Cheng, Q., Johansson, L., Thorell, J.-O., Fredriksson, A., Samén, E., Stone-Elander, S., and Arnér, E. S. J. (2006) ChemBioChem. 7, 1976–1981