Cloning, Overexpression, and Characterization of Glutaredoxin 2, An Atypical Glutaredoxin from *Escherichia coli*

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Glutaredoxin 2 (Grx2) from *Escherichia coli* catalyzes GSH-disulfide oxidoreductions via two redox-active cysteine residues, but in contrast to glutaredoxin 1 (Grx1) and glutaredoxin 3 (Grx3), is not a hydrogen donor for ribonucleotide reductase. To characterize Grx2, a chromosomal fragment containing the E. coli Grx2 gene (grxB) was cloned and sequenced. grxB (645 base pairs) is located between the rimJ and pyrC genes while an open reading frame immediately upstream grxB encodes a novel transmembrane protein of 402 amino acids potentially belonging to class II of substrate export transporters. The deduced amino acid sequence for Grx2 comprises 215 residues with a molecular mass of 24.3 kDa. There is almost no similarity between the amino acid sequence of Grx2 and Grx1 or Grx3 (both 9-kDa proteins) with the exception of the active site which is identical in all three glutaredoxins (Cys-PYC12 for Grx2). Only limited similarities were noted to glutathione S-transferases (Grx2 amino acids 16–72), and protein disulfide isomerasers from different organisms (Grx2 amino acids 70–180). Grx2 was overexpressed and purified to homogeneity and its activity was compared with those of Grx1 and Grx3 using GSH, NADPH, and glutathione reductase in the reduction of 0.7 mM β-hydroxyethyl disulfide. The three glutaredoxins had similar apparent Km values for GSH (2–3 mM) but Grx2 had the highest apparent kcat (554 s⁻¹). Expression of two truncated forms of Grx2 (1–114 and 1–133) which have predicted secondary structures similar to Grx1 (ββαββαβ) gave rise to inclusion bodies. The mutant proteins were resolubilized and purified but lacked GSH-disulfide oxidoreductase activity. The latter should therefore require the participation of amino acid residues from the COOH-terminal half of the molecule and is probably not confined to a Grx1-like NH₂-terminal subdomain. Grx2 being radically different from the presently known glutaredoxins in terms of molecular weight, amino acid sequence, catalytic activity, and lack of a consensus GSH-binding site is the first member of a novel class of glutaredoxins.

Glutaredoxin (Grx) was discovered as a glutathione-dependent hydrogen donor for *Escherichia coli* ribonucleotide reduc-tase (1–3). The first isolated glutaredoxin (glutaredoxin 1) (Grx1), is a 9-kDa protein with two catalytic cysteine residues in the sequence Cys-Pro-Tyr-Cys (4). Apart from its protein disulfide reductase activity with ribonucleotide reductase, Grx1 is also a general GSH-disulfide oxidoreductase, reducing disulfides like β-hydroxyethyl disulfide (HED) in a coupled system with GSH, NADPH, and glutathione reductase (3, 5) (HED assay, Equations 1 and 2),

\[
\text{X-S-S-X} \xrightarrow{\text{Gr}} \text{GSSG + 2X-SH} \quad \text{(Eq. 1)}
\]

\[
\text{GSSG + NADPH + H}^+ \xrightarrow{\text{GR}} \text{2GSH + NADP}^+ \quad \text{(Eq. 2)}
\]

where X-S-S-X is HED and X-SH is β-mercaptoethanol.

In a crude extract of wild-type *E. coli* B, Grx1 constitutes only about 2% of the total GSH-disulfide oxidoreductase activity (HED assay) (5, 6). Later work resulted in the isolation of two additional glutaredoxins which accounted for the major general GSH-disulfide oxidoreductase activity (6). From their order of elution on Sephadex G-50 the new glutaredoxins were called glutaredoxin 2 (Grx2) and glutaredoxin 3 (Grx3). NH₂-terminal sequencing of Grx2 and Grx3 demonstrated that both enzymes contained the typical glutaredoxin motif (Cys-Pro-Tyr-Cys), although Grx2 had an atypical size (M, 27,000) for glutaredoxin. Grx3 (9 kDa) with 82 residues showed 33% sequence identity and a similar secondary structure and tertiary fold (7) as the well characterized Grx1 (8, 9). Thus both Grx1 and Grx3 have the thioredoxin fold (10, 11) and are members of the thioredoxin (Trx) superfamily of disulfide oxidoreductases. In contrast to Grx3, which has about 5% of the catalytic activity of Grx1 as a disulfide reductant of ribonucleotide reductase, Grx2 lacks such activity (6). The relative abundance of Grx2 in *E. coli* (6) makes further investigations of the structure and function of this unknown protein necessary to understand the SH-metabolism in the cell. In this study we have cloned and sequenced the gene coding for Grx2 (grxB) and another gene located immediately upstream grxB coding for a potential transmembrane protein. Grx2 showed large differences to previously known glutaredoxins.

MATERIALS AND METHODS

β-Hydroxyethyl disulfide (HED) was from Aldrich. NADPH and glutathione reductase (yeast) were from Sigma. Diaflo YM10 membranes

1 The abbreviations used are: Grx, glutaredoxin; grxB, gene encoding Grx2; Trx, thioredoxin; HED, β-hydroxyethyl disulfide; ORF, open reading frame; bp, base pair; PAGE, polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography; GST, glutathione S-transferase; PDI, protein disulfide isomerase.
were from Amicon. *E. coli* Grx1 and Grx3, Trx, and thioredoxin reduc- 
tase were purified to homogeneity as described (12). Oligonucleotides 
were synthesized by Pharmacia Biotech Ltd. Restriction enzymes, T4 
DNA ligase, and *Taq* polymerase were from Promega.

**Bacterial Strains and Plasmids—**XL-1 blue and DH15a were used for 
plasmid cloning and DH5a for the cloning of *E. coli* strain G698 (13) was a gift 
Dr. J. McCoy (Genetics Institute, Boston, MA) and was used for over-
expression of Grx2.

**Measurement of Protein Concentration—**Total protein was measured 
using the Bradford protein assay (14) adapted for use on microtiter 
plates. For pure Grx2, a molar extinction coefficient of 21,860 M−1 cm−1 
at 280 nm was used.

**Protein Preparation and Carboxymethylation—**A nearly homogenous 
preparation (more than 90% pure as judged by SDS-PAGE) of *E. coli* 
Grx2 (5 nmol in 50 μl) purified as described (6), was reduced in 0.5 ml 
of 50 mM Tris-Cl, pH 8.0, by incubation for 4 h at 4 °C in the presence 
of 1 mM dithiothreitol. Carboxymethylation was performed by the ad-
dition of 300 μl of 6 M guanidine hydrochloride, 0.4 M Tris-Cl, pH 8.1, 2 
mm EDTA, 5 mM neutralized 14C-iodoacetic acid (Amersham, approxi-
mately 2400 cpm/nmol), and incubation for 4 h at 4 °C. Reagents 
and contaminating proteins were removed by reverse-phase chromatog-
raphy on a C4 (Vydac) HPLC column equilibrated in 0.1% trifluoroacetic 
acid in water and eluted with a linear gradient of acetonitrile (0–40% 
during 60 min, 1 ml/min) containing 0.1% trifluoroacetic acid.

**Activity was expressed as micromoles of NADPH oxidized per min using 
the HED assay for glutaredoxin (100 mM Tris-Cl, pH 8.0, 2 mM 
dithiobis(2-nitrobenzoic acid). This was followed at 412 nm in 100 
mm Tris-Cl, pH 8.0, 0.5 mM 5,5'-dithiobis(2-nitrobenzoic acid) as described (12). *E. coli* thioredoxin was 
used as a positive control.**

**Insulin Reduction by GSH—**Measurements were done at 340 nm 
using the HED assay for glutaredoxin (100 mM Tris-Cl, pH 8.0, 2 mM 
EDTA, 0.1 mM bovine serum albumin, and 6 μg/ml yeast GR was preincubated in 100 mM Tris-Cl, 
2 mM EDTA, pH 8.0 (3). To 500 μl of this mixture in semimicro cuvettes, 
HED was added to a final concentration of 0.7 mM. After 3 min glutare-
doxin was added to the sample cuvettes and the decrease in ΔA280 was 
recorded using a Zeiss PMQ3 spectrophotometer. Activity was expressed 
as micromoles of NADPH oxidized per min using a molar extinction coefficient 
of 6200 M−1 cm−1. Steady-state kinetics measurements were performed in a 
final volume of 100 μl using a Molecular Devices Thermomax microplate reader. Values of ΔA280 were 
multiplied by a factor of 4.3 to give the ΔA400 of a cuvette with a path 
length of 1 cm. All activity measurements were made at 25 °C.

**Determination of SH Groups—**This was done spectrophoto-
metrically with 1 mM 5,5'-dithiobis(2-nitrobenzoic acid) in 200 mM Tris-Cl, pH 8.0, 
6 M guanidine hydrochloride, using a molar extinction coefficient of 
13,600 M−1 cm−1 at 412 nm.

**Assay of Thioredoxin Activity—**The ability of Grx2 to serve as a 
substrate of thioredoxin reductase was determined in the reduction of 
5'-dithiobis(2-nitrobenzoic acid). This was followed at 412 nm in 100 
mm Tris-Cl, pH 8.0, 2 mM EDTA, 0.1 mM bovine serum albumin, 0.2 
mM NADPH, 35 mM *E. coli* thioredoxin reductase, and 0.5 mM 5,5'- 
dithiobis(2-nitrobenzoic acid) as described (12). *E. coli* thioredoxin was 
used as a positive control.

**Insulin Reduction by GSH—**Measurements were done at 340 nm 
using the HED assay for glutaredoxin (100 mM Tris-Cl, pH 8.0, 2 mM 
EDTA, 0.1 mM bovine serum albumin, 0.2 mM NADPH, 1 mM GSH, 
6 μg/ml glutathione reductase) (3) but with bovine insulin (33 μM) in 
place of Trx for their reduction.
or without 1 mM EDTA for 10 min. They were then resuspended in ice-cold 100 mM Tris-Cl, pH 8.0, 1 mM EDTA and left on ice for 10 min. Cells were finally centrifuged and the supernatant was collected as the osmotic shock fraction (13).

**RESULTS**

**Cloning of grxB**—The sequence for the 18 NH$_2$-terminal residues of Grx2 including its active site was determined previously (6). We now digested Grx2 with either Lys-C endoprotease or Asp-N endoprotease and separated the peptides by automated HPLC. Altogether 26 peptides were analyzed by automated

![Characterization of E. coli Glutaredoxin 2](http://www.jbc.org/)
Edman degradation (Fig. 1). Based on the NH₂-terminal sequence and internal peptide K8, primers were designed (N2 and C1) which following PCR resulted in the generation of an 180-bp PCR product containing part of the sequence of grxB. The 180-bp PCR product was used as a probe to screen the Kohara phage library (16) and a positive clone (l233) was identified. After cleavage of lDNA with EcoRI and BamHI a 4.2-kilobase fragment which hybridized with the PCR probe was cloned and sequenced. The novel sequence revealed in the 4.2-kilobase fragment was named grxB region (1943 bp) (Fig. 1) and contained the sequences of grxB and another open reading frame (ORF) upstream grxB.

The 645-bp ORF for grxB encodes a 215-amino acid residue protein with a predicted molecular mass of 24.3 kDa. The deduced amino acid sequence was in agreement with the available amino acid sequence data from the peptides derived from purified Grx2. The NH₂-terminal methionine is coded for by GTG. A consensus ribosomal binding site (GGAGG) is located 6 bases upstream from the first Met residue or 12 bp upstream to an alternative GTG-encoded Met. The TMAP program for the prediction of transmembrane proteins (22) aligned the putative protein to well established transmembrane proteins and a membrane orientation model was constructed (Fig. 3). The grxB region containing grxB and the preceding ORF has been

**Fig. 2. Positioning of the grxB region on the E. coli gene map.** The diagram corresponds to the 4.2-kilobase EcoRI-BamHI fragment in which the grxB region (1943 bp) was found. g203, ORF downstream rimJ; rimJ, gene encoding amino-terminal acetylase of ribosomal protein S5; ORFeqgrxB, gene encoding the putative class II transmembrane antipporter before the gene coding for Grx2; grxB, gene encoding Grx2; ORFqgrxB, ORF before pyrC; pyrC, gene for E. coli dihydroorotase. Unique restriction sites are indicated. Arrows show the 5’ to 3’ reading frame for grxB and the other genes.

**Fig. 3. Membrane orientation model for the protein sequence from ORF-qgrxB.** Boxes represent the putative transmembrane domains. Potential α-helices are numbered 1–12.
submitted to the EMBL data base with accession number X92076.

Expression and Purification of Recombinant Grx2—The gene sequence used for the overexpression of Grx2 was identical to the chromosomal one with the exception that the GTG initiator codon was substituted with ATG to increase expression levels since GTG has been reported as a less efficient start codon compared with ATG (23). Using the overexpression system for Trx fusions (13) with Grx2 replacing Trx, recombinant Grx2 was expressed in a soluble state as high as 50% of total cell protein (Fig. 4). Chromatographies on columns of DE52 cellulose and Sephadex G-50 superfine resulted in a homogeneous protein. A yield of 40% from the purification procedure was obtained. A summary of the purification procedure and yields is shown in Table I. The molecular mass of the recombinant Grx2 was determined as 24.3 kDa by matrix-assisted laser desorption/ionization mass spectrometry. This value is identical to that predicted from the genomic sequence. Peptide sequencing showed that the initiation Met was present in preparations of the recombinant enzyme as was also the case for protein isolated from E. coli cells (6). The specific activity of the recombinant Grx2 (316 units/mg, Table I) is identical to the value published before (6) for the wild type enzyme which was based on a M, of 27,000 rather than the correct value of 24,300.

Grx2 Activity as a GSH-disulfide Oxidoreductase—Previous results (6) suggested that a large part of the GSH-disulfide oxidoreductase activity in E. coli cells measured by the standard HED assay (Equations 1 and 2) is due to Grx2. To compare the steady-state contribution of HED reducing activities of the three E. coli glutaredoxins, we determined their apparent K_m and k_cat values for GSH using the standard HED assay which employs final concentrations of 1 mM GSH and 0.7 mM HED. Comparison was also made with human glutaredoxin (hGrx) (24, 25). Due to the spontaneous reaction between HED and GSH leading to increased NADPH consumption, we only determined rates up to 4.5 mM GSH (Fig. 5). The kinetic data (Fig. 5, Table II) showed that Grx2 had the lowest apparent K_m for GSH and the highest turnover.

Expression and Characterization of Truncated Mutants of Grx2—Sequence alignment of Grx2 with Grx1 and Grx3 showed surprisingly very little homology and identities (Fig. 6). The active site is identical in all three proteins and since Grx2 contains two Cys residues, these must be the catalytic ones located in a similar relative position as in Grx1 and Grx3. Sequence analysis using data base programs revealed some limited homologies to glutathione S-transferases (GSTs) and protein disulfide isomerases (PDIs) (Fig. 6). Since Grx2 seemed to contain Grx-like and PDI-like parts (Fig. 6), we constructed and expressed relevant truncated mutant proteins to examine their possible functional autonomy. The first (amino acids 1–114) mutant corresponded to a part of Grx2 which is long enough to include a Grx-like domain (~80 amino acids). The second mutant was extended to amino acid 133 to include the part with homology to bovine PDI. Both mutants were expressed at high levels but in insoluble states (Fig. 4). Resolution from 8 M urea gave 50% pure preparations but no activity could be detected with the standard HED assay for concentrations of the truncated forms up to 10 μM (assuming a 50% purity, data not shown). In comparison, activity of the least active of the four glutaredoxins, Grx1, is detected readily at concentrations of 0.001 μM.

Grx2 is Not a Substrate for Thioredoxin Reductase—Recombinant alkaline phosphatase can be expressed as an active protein with all its correct disulfides in E. coli trxB mutants, but this cannot happen in trxA mutants lacking thioredoxin (26). This suggests the presence of an alternative substrate for thioredoxin reductase which reduces and inactivates alkaline phosphatase (26). Grx2 did not reduce 5,5′-dithiobis(2-nitrobenzoic acid), demonstrating that it was not a substrate for thioredoxin reductase (data not shown). To examine whether GSH was required for thioredoxin reductase-Grx2 interactions, standard HED assays were carried with thioredoxin reductase substituting for glutathione reductase. Grx2 was not reduced under these conditions as measured at 340 nm showing that it did not react with thioredoxin reductase through GSH.

Intracellular Localization of Grx2—To examine the intracellular localization of Grx2, periplasmic and cytosolic fractions of non-transformed E. coli cells and transformants were analyzed by SDS-PAGE (Fig. 8). Grx2 was clearly cytosolic. The enzyme was not released (even when overexpressed) by osmotic shock conditions which is different from Trx (1, 13) and Grx1 (1).

Table I

<table>
<thead>
<tr>
<th>Purification of recombinant Grx2 from 6.2 g of E. coli GH698-pe1Grx2 cells</th>
<th>Proteina</th>
<th>Volume</th>
<th>Activityb</th>
<th>Total activity</th>
<th>Specific activity</th>
<th>Yield</th>
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<tr>
<td>Step</td>
<td>mg</td>
<td>ml</td>
<td>units/ml</td>
<td>units</td>
<td>units/mg</td>
<td>%</td>
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<tr>
<td>Cell lysate supernatant</td>
<td>695</td>
<td>57</td>
<td>1283</td>
<td>73,133</td>
<td>105</td>
<td>100</td>
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<tr>
<td>DE52 pool (concentrated)</td>
<td>208</td>
<td>3</td>
<td>14,122</td>
<td>42,367</td>
<td>204</td>
<td>58</td>
</tr>
<tr>
<td>G50 pool</td>
<td>141</td>
<td>12</td>
<td>2455</td>
<td>29,462</td>
<td>210</td>
<td>40</td>
</tr>
</tbody>
</table>

* Protein was determined by the method of Bradford (14).

* One unit of activity is 1 μmol of NADPH oxidized per min in the standard HED assay (3).

* The specific activity of purified Grx2 was 316 units/mg using A 280 values for Grx2 and a molar extinction coefficient of 21,880 M⁻¹ cm⁻¹.
Characterization of E. coli Glutaredoxin 2

This paper describes the cloning and sequence determination of a genomic region containing grxB and presents findings on the characterization of recombinant Grx2. Previously two other glutaredoxins from E. coli, both 9-kDa proteins, were shown to have the same active site sequence Cys-Pro-Tyr-Cys and to catalyze GSH-disulfide oxidoreductions via a GSH-binding site (7, 27). Grx2 contains the same active site sequence Cys-Pro-Tyr-Cys as the other two glutaredoxins and is highly active in the general GSH-disulfide oxidoreductase assay with the small disulfide HED as a substrate. Grx2, however, has a larger size and lacks activity as a hydrogen donor (protein disulfide reductase) for ribonucleotide reductase (6). Alignment of Grx2 with other proteins showed no further extensive homology to known glutaredoxins or thioredoxins (Fig. 6). Grx2 therefore lacks the conserved sequences of Grx1 and Grx3 defining a GSH-binding site previously experimentally verified by NMR for the mixed disulfide between E. coli Grx1 C14S and GSH (28). Amino acid residues of Grx2 that may be the homologues to amino acids of Grx1 involved in binding to GSH (underlined) are Y11 (Y13 in Grx1), QVP69 (TVP69 in Grx1), and YVD70 (YTD74 in Grx1).

Data base searches showed that Grx2 was weakly homologous to sequences of two GSH S-transferases (GSTs) (Grx2 amino acids 47–69 and 86–112), a relative of GST, crystallin SL-11 (28) (Grx2 amino acids 16–71), and PDIs of different organisms (70–180) (Fig. 6). The significance of this is unknown. Secondary structure prediction using the method of Rost and Sander (29) suggested that the first third of Grx2 (2–70) contained an alternation of β-sheets and α-helices (ββαββ), while the remaining part (84–212) was predominately helical (Fig. 6). The ββαββ prediction for the NH2-terminal third of the molecule is similar to the characteristic ββαββα secondary structure of the thioredoxin fold found in E. coli Trx (10), Grx1 (8), Grx3 (7), glutathione peroxidase (30), and GSH S-transferases (GST) (31). In the latter a first Trx-like domain (−80 amino acids) is connected (through a short linker of 8 amino acids for GSTμ1 (32) to a second domain (−130 amino acids) composed of α helices (31). Although the amino acid sequence of Grx2 was markedly different from other members of the Trx superfamily or GSTs including the known E. coli GST (Fig. 6) (33), the NH2-terminal third of the molecule may have the thioredoxin fold. E. coli DekB, for example, has almost no sequence homology to Trx (34) but contains a Trx-like domain. To examine whether Grx2 contained a fully active Trx/Grx-like domain at its NH2 terminus, we overexpressed two truncated mutant proteins. The first (1–114) included the putative ββαββα fold and the second (1–133) included a part with homology to bovine PDI (Fig. 6). Both overexpressed pro-

**TABLE II**

Activity of different glutaredoxins and their properties

<table>
<thead>
<tr>
<th>Molecular mass</th>
<th>(K_m)</th>
<th>(k_{cat})</th>
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</thead>
<tbody>
<tr>
<td>Grx1</td>
<td>9.7</td>
<td>3.0 ± 0.7</td>
</tr>
<tr>
<td>Grx2</td>
<td>24.3</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Grx3</td>
<td>9</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>hGrx</td>
<td>12</td>
<td>1.9 ± 0.2</td>
</tr>
</tbody>
</table>

**FIG. 5.** Comparison of relative activity of Grx1, Grx2, Grx3, and human Grx (hGrx) (24, 25) with GSH in the GSH-disulfide oxidoreductase assay. The conditions of the assay (with 0.7 mM HED) were identical to those previously described (3) with the exception that concentrations of GSH were varied as indicated in the samples containing glutaredoxin and their respective controls. Turnover represents the rate of NADPH oxidized/s by 1 mol of each glutaredoxin. Activity was calculated after subtracting the spontaneous reduction rate observed in the absence of glutaredoxin. Three separate measurements were made for each glutaredoxin and their respective controls. Turnover represents the mean value is shown. Error bars represent ±1 S.E.

**FIG. 6.** Sequence alignment of Grx2 and prediction of secondary structure. The alignment of Grx2 with Grx1, Grx3, and E. coli GST (GST Eco) was performed using the on-line software facility of Genome Ecole pour les Etudes et de la Recherche en Informatique et Electronique. The alignment of Grx2 with the different PDI was made using the BLAST program software of the National Center for Biotechnology Information. Secondary structure information was obtained using the secondary structure prediction software of Rost and Sander (29). L, prediction for loop; α, prediction for α-helix; β, prediction for β-pleated sheet structure. Alignment to GST from Pseudomonas (GST Ps), GST 6.0, and S-cystatin SL11 was performed using the TMAP program (22). Identical amino acids are boxed.
Characterization of E. coli Glutaredoxin 2

![Figure 7](http://www.jbc.org/)

**Figure 7.** GSH-dependent reduction of insulin by glutaredoxins. Insulin, 30 μM, was used in place of 0.7 mM HED in the GSH-disulfide oxidoreductase assay (see "Materials and Methods"). The concentrations of glutaredoxins were varied and activity is expressed as oxidation of NADPH at 340 nm.

![Figure 8](http://www.jbc.org/)

**Figure 8.** Preparation of different E. coli cell fractions to localize Grx2. Fractions representing equal cell numbers were loaded on a reducing 10% SDS-PAGE gel. PPCL, cell lysate without periplasmic fraction; PF, periplasmic fraction; SW, supernatant of 20% sucrose wash (osmotic shock); TE, supernatant in 100 mM Tris-Cl, pH 8.0, 1 mM EDTA following sucrose wash; OSCL, cell lysate after osmotic shock without SW and TE fractions; T, supernatant in 100 mM Tris-Cl, pH 8.0 following sucrose wash; TCLS, total cell lysate supernatant; 8M US, insoluble material corresponding to the TCLS, resolubilized in 8 M urea, 100 mM dithiothreitol, pH 8.0, 1 mM EDTA.

The catalytic activity of recombinant Grx2 and three other classical glutaredoxins was compared in the GSH-disulfide oxidoreductase assay varying the concentrations of GSH with the HED concentration constant (0.7 mM). All the different glutaredoxins had similar apparent Michaelis constants (Km) but Grx2 showed the highest apparent turnover number (kcat.

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4 R. Morgenstern, Karolinska Institute, Department of Toxicology, personal communication.
involved in GSH-dependent thiol-disulfide exchange reactions making disulfides in proteins. Such reactions and other potential physiological roles linked to the transporter can be addressed by gene inactivation experiments currently in progress.

Acknowledgments—We are grateful to Carina Jonsson for excellent technical assistance, Valentina Bonetto for the determination of the molecular mass of recombinant Grx2 by matrix-assisted laser desorption/ionization mass spectrometry, and Dr. Ralf Morgenstern for the technical assistance, Valentina Bonetto for the determination of the molecular mass of recombinant Grx2 by matrix-assisted laser desorption/ionization mass spectrometry, and Dr. Ralf Morgenstern for the TNB and CDNB assays. The generous help of Malin Rohdin is also acknowledged.

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