The Putative Glutathione Peroxidase Gene of Plasmodium falciparum Codes for a Thioredoxin Peroxidase*

Received for publication, September 20, 2000, and in revised form, October 27, 2000
Published, JBC Papers in Press, November 21, 2000, DOI 10.1074/jbc.M008631200

Helena Sztajer‡, Benoit Gamain§, Klaus-Dieter Aumann‡, Christian Slomianny¶, Katja Becker**, Regina Brigelius-Flohé‡‡, and Leopold Flohé‡§§

From the ‡Department of Biochemistry, Technical University of Braunschweig, Mascheroder Weg 1, 38124 Braunschweig, Germany, §NAID, National Institutes of Health, Bethesda, Maryland 20892, ¶National Centre for Biotechnology, Mascheroder Weg 1, 38124 Braunschweig, Germany, ‡‡University of Science and Technology Lille, INSEERM EPI 9938, Batiment SN3, 59655 Villeneuve d’Ascq Cedex-France, **Research Center for Infectious Diseases, Würzburg University, Röntgenring 11, 97070 Würzburg, Germany, and §§German Institute of Human Nutrition, Arthur-Scheunert-Allee 114-116, D-14558 Bergholz-Rehbrücke, Germany

A putative glutathione peroxidase gene (Swiss-Prot accession number Z 68200) of Plasmodium falciparum, the causative agent of tropical malaria, was expressed in Escherichia coli and purified to electrophoretic homogeneity. Like phospholipid hydroperoxide glutathione peroxidase of mammals, it proved to be monomeric. It was active with \( \text{H}_2\text{O}_2 \) and organic hydroperoxides but, unlike phospholipid hydroperoxide glutathione peroxidase, not with phosphatidylcholine hydroperoxide. With glutathione peroxidases it shares the ping-pong mechanism with infinite \( V_{\text{max}} \) and \( k_{\text{cat}} \) when analyzed with GSH as substrate. As a homologue with selenocysteine replaced by cysteine, its reactions with hydroperoxides and GSH are 3 orders of magnitude slower than those of the selenoperoxidases. Unexpectedly, the plasmodial enzyme proved to react faster with thioredoxins than the selenoperoxidases. Encoding a sulfur homologue of the mammalian selenoproteins, the gene product should be substantially less efficient than the host cell enzymes. (12, 13). The comparatively high sensitivity of Plasmodia species to hydroperoxides could therefore result from the necessity to rely on sulfur-catalyzed hydroperoxide reduction, whereas the host cell makes use of the more efficient selenium catalysis. Beyond, the amino acid sequence deduced from the plasmodial GPx gene resembles phospholipid hydroperoxide peroxidase (PHGPx, GPx-4), which displays a degenerate substrate specificity (14). Correspondingly, it could not be taken for granted that the plasmodial GPx homologue is indeed a glutathione peroxidase. The plasmodial GPx gene was therefore heterologously expressed in Escherichia coli, and the protein was purified in sufficient quantities to allow an in-depth functional analysis. As expected, it proved to be a peroxidase acting on a broad spectrum of hydroperoxides with low efficiency. Also, the reaction rates with GSH were surprisingly low. Instead, thioredoxins reduced the enzyme efficiently enough to reclassify this member of the glutathione peroxidase family as a thioredoxin peroxidase and to substitute the acronym PTPx for the originally introduced glutathione peroxidase of \( P. \ falciparum \) (PHGPx) (2).

EXPERIMENTAL PROCEDURES

Heterologous Expression and Purification of PTPx—The full-length cDNA encoding the PTPx was amplified by reverse transcriptase-polymerase chain reaction using asynchronous blood stage RNA and
cloned into the pET5a vector (Calbiochem-Novabiochem) between the EcoRI and NdeI sites and transformed into E. coli BL21 (DE3) pLysS.

The clone was grown in carbenicillin (1 mg/ml)-supplemented LB media at 37 °C and 180 rpm to an A600 of 0.5 and induced with 0.4 mM isopropyl-1-thio-β-D-galactopyranoside. The culture was grown for an additional 5 h and controlled for expression by SDS-polyacrylamide gel electrophoresis. For routine preparation, cells were harvested 3 h after induction, resuspended in 50 mM Tris, 1 mM dithiothreitol, pH 8.5 (buffer A), disrupted with a French press at the 900 psi, and centrifuged at 18,000 rpm for 10 min. The supernatant was loaded on a Macro-Prep® High Q Support fitted on a Macro-Prep® High S Support column with a flow rate of 2 ml/min (Bio-Rad). The column was washed with 10 bed volumes of buffer A. The Macro Prep® High S Support column alone was eluted with a NaCl gradient (0–2 M) in the same buffer. Fractions with GPx activity were concentrated by ultrafiltration (Omega Cell, cut-off 10 kDa, Fall Gelman Sciences, An Arbor, MI), loaded onto a Sephacryl S-200 (Amersham Pharmacia Biotech) gel filtration column equilibrated with 0.1 M Tris, 0.1 M NaCl, 5 mM EDTA, pH 7.6, and eluted at a flow rate of 0.5 ml/min. Active fractions were analyzed by SDS-polyacrylamide gel electrophoresis for homogeneity and stored at 4 °C.

**Characterization of Expression Product** —The molecular mass of de-natured PfTPx was estimated by silver-stained (15%) SDS-polyacrylamide gel electrophoresis using the Phast System® (Amersham Pharmacia Biotech) with an acrylamide gradient of 8–25% and a 10-kDa ladder as reference. The molecular mass of native PfTPx was estimated by gel filtration on a Sephadex S-100 column equilibrated with a 0.1 M Tris buffer of pH 7.6 containing 5 mM EDTA at a flow rate of 0.5 ml/min. Chymotrypsinogen, bovine serum albumin, blue dextran, and cytochrome c were co-chromatographed as reference proteins. Protein concentration was determined according to Bradford (16) with the reagent from Bio-Rad, taking bovine serum albumin as the standard. The precise molecular weight was determined by matrix-assisted laser desorption and ionization time of flight (MALDI-TOF) mass spectrometry with a Bruker Reflex II-MALDI-TOF mass spectrometer (Bruker-Franzen-Analytik, Bremen, Germany). For this purpose the protein was precipitated with trichloroacetic acid, washed with the acetone, and resuspended in saturated matrix solution (10 mg/ml sinapinic acid in 40% acetonitrile and 0.1% trifluoroacetic acid). The protein at a final concentration about 50 pmol/μl matrix solution was accelerated at 20 kV.

Spectra were externally calibrated using bovine serum albumin as standard. Approximately 200 shots were summed for each spectrum. N-terminal sequencing was performed with an Applied Biosystems 494 A sequencer.

**Activity Measurement and Kinetic Analysis**—GPx activity was measured by monitoring the glutathione reductase-catalyzed NADPH oxidation at 340 nm at 30 °C in 0.5 ml containing 0.3 mM NADPH in 0.1 M Tris, pH 7.6, 0.1% Triton, 5 mM EDTA, 3.3 mM GSH, and 73 μM t-butylnitroperoxide (t-bNOOH) (17). Specificity for the hydroperoxide substrate was investigated with t-BOOH (Merck), cumene hydroperoxide (Merck, Eurolab, Darmstadt, Germany), H2O2 (Sigma-Aldrich), and phosphatidylcholine hydroperoxide prepared according to Maiorino et al. (18). The concentration of hydroperoxides was determined by allowing the GPx reaction to run to completion. For quantification of t-BOOH, H2O2, and cumene hydroperoxide bovine GPx (Sigma-Aldrich), for phosphatidylcholine hydroperoxide a PHGPx preparation of rat testis (19) was used.

Thioredoxin peroxidase activity was determined analogously by replacing GSH by thioredoxin and glutathione reductase by thioredoxin reductase. In each case the particular thioredoxin was coupled to the plasmodial enzyme is a member of the GPx superfamily, yet is more related to PHGPx than to any of the other GPx types (Fig. 2) displaying 64, 55, 51, and 45 identities with PHGPx, cGPx, gastrointestinal GPx, and pGPx, respectively. Also the PfTPx sequence as obtained by heterologous expression in E. coli, corresponds to the homologous stretch of 170 amino acid residues found in mature porcine PHGPx (24). Like PHGPx, PfTPx proved to be monomeric when subjected to Sephadex G-100 gel filtration under nondenaturing conditions. The activity of the eluting enzyme was verified by GPx activity measurements. It eluted with an apparent molecular mass of 18.9 kDa, whereas no activity could be detected in the fractions corresponding to the molecular mass of its tetrameric congeners, pGPx or cGPx. As is evident from the tertiary structures of cGPx (25) and pGPx (26), subunit contact surfaces in these tetrameric GPx types are essentially built up by residues corresponding to the inserts at positions 121 and 161 of the PfTPx sequence (27). They are missing in both PfTPx and PHGPx, respectively.

**RESULTS**

**Comparison of PfTPx with cGPx and PHGPx**—Alignment of PfTPx with mammalian glutathione peroxidases reveals that the plasmodial enzyme is a member of the GPx superfamily yet is more related to PHGPx than to any of the other GPx types (Fig. 2) displaying 64, 55, 51, and 45 identities with PHGPx, cGPx, gastrointestinal GPx, and pGPx, respectively. Also the PfTPx sequence as obtained by heterologous expression in E. coli, corresponds to the homologous stretch of 170 amino acid residues found in mature porcine PHGPx (24). Like PHGPx, PfTPx proved to be monomeric when subjected to Sephadex G-100 gel filtration under nondenaturing conditions. The activity of the eluting enzyme was verified by GPx activity measurements. It eluted with an apparent molecular mass of 18.9 kDa, whereas no activity could be detected in the fractions corresponding to the molecular mass of its tetrameric congeners, pGPx or cGPx. As is evident from the tertiary structures of cGPx (25) and pGPx (26), subunit contact surfaces in these tetrameric GPx types are essentially built up by residues corresponding to the inserts at positions 121 and 161 of the PfTPx sequence (27). They are missing in both PfTPx and PHGPx, which explains their monomeric nature.

PfTPx, like PHGPx, was more active with cumene hydroperoxide, less active with H2O2 and t-BOOH, and unlike PHGPx, did not at all accept phosphatidylcholine hydroperoxide (data
not shown). In terms of molar efficiencies, PfTPx appeared markedly poorer than bovine cGPx coinvestigated as a selenoperoxidase reference standard. Specific GPx activities, as measured under routine conditions, differed by three orders of magnitude (not shown).

The kinetic mechanism for GSH-dependent hydroperoxide reduction by PfTPx was evaluated by means of the single curve progression analysis (22) at various fixed GSH concentrations, which were kept constant over time by regeneration, and a suboptimal concentration of ROOH, which declined over time and correspondingly lead to slowing down of the reaction rate. From these curves, the reciprocal concentrations of ROOH at intervals of 2 s were derived and plotted against the initial velocities at each pertinent time point, as exemplified for the turnover of t-bOOH by GSH in Fig. 3. The data are presented as Dalziel plots (23), in which the reciprocal velocities are multiplied by enzyme molarities to facilitate the extrapolation of meaningful kinetic coefficients, as indicated. As shown in Fig. 3, such primary plots yielded parallel slopes for different concentrations of GSH, as is typical for "enzyme substitution" or "ping-pong" mechanisms. Repplotting the reciprocal GSH concentrations against the reciprocal apparent $V_{\text{max}}$ for infinite concentrations of t-BOOH yielded a straight line cutting at the ordinate origin (Fig. 4). The same kinetic pattern was displayed by PfTPx with H$_2$O$_2$ and cumene hydroperoxide as long as GSH was used as reducing substrate (Table I). It can be described by a Dalziel equation for two substrate ping-pong mechanisms.

$$\frac{[E_0]}{V} = \Phi_1 + \frac{\Phi_2}{[\text{ROOH}]} + \frac{\Phi_3}{[\text{GSH}]} \quad \text{(Eq. 1)}$$

wherein the coefficient $\Phi_3$ approximates zero. Accordingly, limiting $V_{\text{max}}$ and $K_m$ values are infinite. Such lack of enzyme saturation is due to two distinct catalytic phenomena, either the formation of enzyme-substrate complex is slower than the reaction within the complexes or specific enzyme-substrate complexes not formed at all, as is presumed in Equations 2–4.

$$E_{\text{red}} + \text{ROOH} \rightleftharpoons E_{\text{red}} + \text{ROH} \quad \text{(Eq. 2)}$$

$$E_{\text{red}} + \text{GSH} \rightleftharpoons E - \text{SG} + \text{H}_2\text{O} \quad \text{(Eq. 3)}$$

$$E - \text{SG} + \text{GSH} \rightleftharpoons E_{\text{red}} + \text{GSSG} \quad \text{(Eq. 4)}$$

In this case, the coefficient $\Phi_1$ is defined as the reciprocal rate constant $k_{-1}$ for the net forward reaction of reduced enzyme with ROOH and depends on the nature of the peroxide (Table I). $k_{-1}$, is defined as $k_{-2} - k_{-3}$, and may be regarded as $k_{-1}$, since the partial reaction shown in Equation 2 should be irreversible. $\Phi_2$ is the reciprocal $k_{-2}$ for the two-step regeneration of the reduced enzyme by GSH according to Equations 3 and 4. Therefore, the physical meaning of $k_{-2}$ is more complex (Equation 5).

$$k_{-2} = k_{-2} - k_{-3} + k_{+3} - k_{+3} \quad \text{(Eq. 5)}$$

The kinetic pattern of PTPxs is identical to that of the selenium-containing glutathione peroxidases (28–31). However, the kinetic coefficients $\Phi_1$ and $\Phi_2$ of PTPxs differ markedly from those of the selenoproteins. Instead, they are similar to those of a sulfur homologue of PHGPx produced by site-directed mutagenesis. Although $\Phi_1$, for authentic porcine PHGPx is 0.07 mM s, and $\Phi_2$, is 8.3 mM s, the cysteine-containing PHGPx murein, with a $\Phi_1$ of 20 and $\Phi_2$ of 40,000 mM s (13), resembles PTPx.

**Thioredoxin as Reducing Substrate of PTPxs**—In view of the
low efficiency of PfTPx in GSH-dependent hydroperoxide reduction and the sequence similarities to the less GSH-specific pGpx (32) and PHGpxs (33, 34), its activity with alternative reducing substrates, notably thioredoxins, was investigated. Table II shows that PfTPx indeed accepts thioredoxins of various species. The turnover rates observed with thioredoxin of E. coli and man in the submillimolar range were similar to those measured with 10 mM GSH, and autologous plasmodial thioredoxin also working with sulfur catalysis (44). These observations suggest that, with sulfur catalysis, rate constants near $10^4 \text{ M}^{-1} \text{s}^{-1}$ can be reached for the reduction of hydroperoxides by thiols, whereas $10^2 \text{ M}^{-1} \text{s}^{-1}$ are commonly observed with selenium catalysis. Rate constants beyond $10^6 \text{ M}^{-1} \text{s}^{-1}$ are also reported for heme-catalyzed hydroperoxide reduction (45). Less efficient hydroperoxidases can reasonably be implicated in an-

**DISCUSSION**

The glutathione peroxidase family of proteins is spread over the whole living kingdom (2, 14). The name-giving classical glutathione peroxidase, however, has so far only been detected in vertebrates, where it proved to be a selenoprotein (35, 36) and of the related selenoproteins, pGpxs, gastrointestinal GPxs, and PHGpxs, only the latter has been identified in a nonvertebrate species, Schistosoma mansoni (37, 38). Genes encoding homologous proteins in which the active site selenocysteine is replaced by cysteine appear to be widely distributed in nature (2, 14). Such proteins are commonly addressed as glutathione peroxidases, although their activity has never been systematically analyzed. In fact, some representatives of the family have been discovered in a biological context not reminiscent of an antioxidant function, e.g., the cohalmine-binding protein in E. coli (39), the salt stress-responsive protein in Citrus plants (40), and androgen-responsive epidermal proteins in mammals (41). Furthermore, any relevant peroxidase activity of such proteins may be questioned considering the low efficiencies of cysteine-containing muteins of GPxs and PHGpxs.

To our knowledge, the efficiency and specificity of a naturally occurring nonselenium GPx homologue is addressed here for the first time. To this end, we have unfortunately to rely on an heterologously expressed protein, since a purification of PfTPx from P. falciparum has not yet been feasible. In its basic characteristics, however, the PfTPx gene expression product, as here obtained, is presumed to closely resemble authentic PfTPx. Admittedly, the discrepancy between the size of the deduced maximum sequence and the isolated protein is substantial. But this is also observed with the closely related mammalian PHGpx. As in the PfTPx gene, two potential start codons are contained in the PHGpx gene. In this case they are alternatively used in a tissue-specific manner, either leading to mitochondrial or cytosolic localization of the enzyme (42, 43). In each case the processed expression product is the same (43), i.e., a protein of about 19 kDa corresponding in size to a PfTPx starting with Met-26. Monitoring the production of PfTPx in E. coli does not reveal any primary product in the 24-kDa region. The electrophoretic mobility of the band showing up upon induction is identical to that of the isolated product (Fig. 1). Obviously, our E. coli production strain has chosen the second ATG start codon, which is also the preferred start codon of the mammalian PHGpx genes. Certainly, the heterologously expressed PfTPx contains all residues constituting the catalytic triad essential for hydroperoxide reduction, i.e., Cys-76, Gln-111, and Trp-169 in homologous position to Gln-81, selenocysteine 46, Trp-136 of porcine PHGpx (13, 24). Whether the missing N-terminal extension and the minor C-terminal truncation affects specificity remains to be established. The gain of a new specificity, as described here, is not likely explained by such modifications.

The low efficiency of PfTPx in reducing hydroperoxides does not surprise but raises the question whether such low efficiency peroxidases may be implicated in antioxidant defense at all. The $k_{-1}$ values for the reaction of PfTPx with t-bOOH, cumene hydroperoxide, and H$_2$O$_2$ reported here come close to the $k_{-1}'$ of the cysteine mutein of porcine PHGpx with phosphatidylcholine hydroperoxide (13). Similar $k_{-1}'$ values were reported for tryptaredoxin peroxidase, a structurally unrelated peroxiredoxin also working with sulfur catalysis (44). These observations suggest that, with sulfur catalysis, rate constants near $10^4 \text{ M}^{-1} \text{s}^{-1}$ can be reached for the reduction of hydroperoxides by thiols, whereas $10^2 \text{ M}^{-1} \text{s}^{-1}$ are commonly observed with selenium catalysis. Rate constants beyond $10^6 \text{ M}^{-1} \text{s}^{-1}$ are also reported for heme-catalyzed hydroperoxide reduction (45). Less efficient hydroperoxidases can reasonably be implicated in an-

**FIG. 4.** Secondary Dalziel plot for the PfTPx-catalyzed reduction of t-bOOH by GSH. The reciprocal apparent maximum velocities (n = 3 each) extrapolated for infinite concentration of t-boOH, are plotted against the reciprocal GSH concentrations. The slope yields $\Phi_2$. Cutting the ordinate at zero indicates that the term $\Phi_1$ approaches zero, which implies that the maximum velocity and $K_m$ values of PfTPx are infinite for the pair of substrates investigated.
The Dalziel coefficients $\Phi_1$ and $\Phi_2$ of PfTPx are calculated from three different sets of experiments. The extrapolated coefficient $\Phi_1$ is zero within experimental error for all reactions. $\Phi_2$ values for the GSH-dependent reaction of PfTPx do not differ significantly, whereas $\Phi_1$ and $k_2$ values depend on the nature of the hydroperoxide. CumeneOOH, cumene hydroperoxide; PCOOH, phosphatidylcholine hydroperoxide; Sec, selenocysteine. Data for PHGPx are taken from Maiorino et al. (13).

| Enzyme   | Substrate | $\Phi_0$ | $\Phi_1$ | $k_1'$ | $\Phi_2$ | $k_2'$ |
|----------|-----------|----------|----------|--------|----------|--------|
| PfTPx    | t-bOOH    | 0        | 327.8 ± 24.7 | $3.3 \times 10^3$ | 43,100 ± 9046 | 23     |
| PfTPx    | CumeneOOH | 0 (0.8)  | 50.0 ± 23.1  | $2.0 \times 10^4$ | 45,539 ± 2589 | 22     |
| PfTPx    | H_2O_2    | 0        | 398.6 ± 14.2 | $2.5 \times 10^3$ | 52,600 ± 3786  | 19     |
| PHGPx    | PCOOH     | 0        | 0.07      | $1.4 \times 10^7$ | 8.4        | 1.2 × $10^5$ |
| PHGPx    | Sec → Cys | 0        | 20.0      | $5.0 \times 10^4$ | 40,000     | 25     |

GSH as reducing substrate was replaced by Trx of $E. coli$ (85 μM), human (200 μM), and $P. falciparum$ (5 μM), and glutathione reductase as indicator enzyme (5.6 units/ml) was replaced by the autologous thioredoxin reductases (0.5 unit/ml, 0.56 unit/ml, 0.001 unit/ml, respectively). With each of the thioredoxins, the specific rate was at least as high as that observed with GSH at 50–2000 × the concentration.

| Donor substrate | Specific rate |
|-----------------|--------------|
| PfTrx (5.0 μM)  | 0.47         |
| Human Trx (200 μM) | 0.12       |
| $E. coli$ Trx (85 μM) | 0.16     |
| GSH (10 mM)      | 0.10         |

Fig. 5. Representative primary Dalziel plot of the PfTPx-catalyzed reduction of t-bOOH by PfTrx. As in the GSH-dependent reaction, parallel lines are observed with 1.5 (■), 2.5 (▲), and 3.5 (▼) mM PfTrx, indicating a ping-pong mechanism. The slopes ($\Phi$) do not differ significantly from those obtained with GSH as reducing substrate.

By means of the rate equation (Equation 1) and the kinetic coefficients (Table III), the PfTrx-driven H_2O_2 reduction reach apparent maximum velocities in the μM range of PfTrx. With 10 mM GSH, which may be taken as an upper physiological concentration, the GSH-dependent reduction of t-bOOH by PfTrx does not differ significantly from those obtained with GSH as reducing substrate.

Nevertheless, a competition of GSH with thioredoxin for oxidized PfTPx under in vivo conditions cannot be fully ruled out. Irrespective of uncertainties about the concentrations of GSH and Trx in the various differentiation states of the parasites, the kinetic parameters of PfTPx imply that the GSH-driven reaction falls short under most conditions that could be envisaged to be physiologically relevant, as is easily calculated by means of the rate equation (Equation 1) and the kinetic coefficients (Table III). The PfTrx-driven H_2O_2 reduction reaches apparent maximum velocities in the μM range of PfTrx. With 10 mM GSH, which may be taken as an upper physiological concentration, the GSH-dependent reduction of t-bOOH by PfTrx does not differ significantly from those obtained with GSH as reducing substrate.

PLASMODIAL THIOREDOXIN PEROXIDASE

**TABLE I**

Kinetic coefficients and apparent rate constants of PfTPx for the reduction of different hydroperoxides by GSH

Table I gives representative rate constants of PfTPx for the reduction of different hydroperoxides by GSH.

**TABLE II**

Specificity of PfTPx for thioredoxins

Table II shows the specificity of PfTPx for thioredoxins.

**TABLE III**

Kinetic coefficients and apparent rate constants of PfTPx for the reduction of different hydroperoxides by GSH

The kinetic coefficients and apparent rate constants of PfTPx for the reduction of different hydroperoxides by GSH are given in Table III.

**FIG. 6.** Secondary Dalziel plot of the PfTPx-catalyzed reduction of t-bOOH by PfTrx. Data are based on duplicates of primary plots, as exemplified in Fig. 7, plus two pilot measurements performed at 5 μM PfTrx. The line cuts the ordinate at $\Phi_0$, which adopts a positive value with PfTrx as the reducing substrate.

**FIG. 5.** Representative primary Dalziel plot of the PfTPx-catalyzed reduction of t-bOOH by PfTrx. As in the GSH-dependent reaction, parallel lines are observed with 1.5 (●), 2.5 (▲), and 3.5 (▼) mM PfTrx, indicating a ping-pong mechanism. The slopes ($\Phi$) do not differ significantly from those obtained with GSH as reducing substrate.

tioxidant defense if their low molar efficiency is compensated for by extreme concentration, as has been proposed for the hydroperoxide detoxification by a peroxiredoxin in trypanosomes (44). In line with these considerations, $E. coli$ overexpressing PfTPx proved to be slightly more resistant to oxidative challenge, which is not surprising in view of PfTPx being the prominent protein in such cells (Fig. 1). At less abundant levels, the ability of PfTPx to balance oxidative stress may be doubted.

The observation that a GPx homologue reacts with thiols other than GSH is not surprising either. Only for the cytosolic GPxs has a pronounced specificity for GSH been documented (46). This specificity is considered to be due to basic residues, Arg-57, Arg-102, Arg-184, Arg-185, and Lys-92 in bovine cGPx, which direct the SH group of the substrate to the active-site selenium atom by electrostatic forces (47). These residues are only partially conserved in the gastrointestinal and extracellular isozymes and completely lost in PHGPx-type enzymes. Accordingly, pGPx has been reported to accept thioredoxin and glutaredoxin (32), and PHGPx, in the absence of GSH, can form high molecular weight protein aggregates that are cross-linked by Se-S and/or S-S bridges, a process shown to be of physiological importance in late phases of mammalian sperm maturation (33). It does, however, not react with $E. coli$ thioredoxin and human thioredoxin 1 and 4.3 In contrast, PfTPx appears to be specialized for interaction with thioredoxin, as evident from the kinetic data reported.

2 B. Gamain, unpublished observations.

3 Brigelius-Flohé and L. Flohé, unpublished data.
For the GSH-driven reaction, $\Phi_2$ values were averaged from all experiments irrespective of the peroxide substrate. $\Phi_s$ value and $k_v'$ values are those for t-BOOH.

| Donor substrate | $\Phi_0$ | $k_{cat}$ | $\Phi_s$ | $k_v$ | $\Phi_2$ | $k_v'$ | $K_m$ REDSH |
|-----------------|---------|-----------|---------|-------|--------|--------|-----------|
| GSH             | 1.0     | $328 \pm 25$ | $3 \times 10^3$ | 47.029 | 21.2   | $5 \times 10^4$ | 10.3      |
| PfTrx           | 1.8     | 0.55      | $383 \pm 29$ | $2.6 \times 10^3$ | 18.5   | $5 \times 10^4$ | 10.3      |
47. Aumann, K.-D., Bedorf, N., Brigelius-Flohe, R., Schomburg, D., and Flohe, L. (1997) Biomed. Environ. Sci. 10, 136–155
48. Flohe, L. (1979) CIBA Found. Symp. 65, 95–122
49. Brigelius-Flohe, R., Friedrichs, B., Maurer, S., Schultz, M., and Streicher, R. (1997) Biochem. J. 328, 199–203
50. Jin, D.-Y., Chae, H.-Z., Rhee, S.-G., and Jeang, K.-T. (1997) J. Biol. Chem. 272, 30952–30961
51. Flohe, L., Andreesen, J. R., Brigelius-Flohe, R., Maiorino, M., and Ursini, F. (2000) IUBMB Life 49, 411–420
52. Gamain, B., Arnaud, J., Favier, A., Camus, D., Dive, D., and Slomianny, C. (1996) Free Radic. Biol. Med. 21, 559–565
53. Chen, J.-W., Dodia, C., Feinstein, S. I., Jain, M. K., and Fisher, A. B. (2000) J. Biol. Chem. 275, 28421–28427
54. Andreesen, J. R., Wagner, M., Sonntag, D., Kohlstock, M., Harms, C., Gurzinsky, T., Jäger, J., Parther, T., Kabisch, U., Grantzdörffer, A., Fleh, A., and Söhl, B. (1999) Biofactors 10, 263–270