ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 7 & 8, 2006
© Mary Ann Liebert, Inc.

Forum Review

Thioredoxin Networks in the Malarial Parasite *Plasmodium falciparum*

CHRISTINE NICKEL, STEFAN RAHLFS, MARCEL DEPONTE, SASA KONCAREVIC, and KATJA BECKER

ABSTRACT

The intraerythrocytic protozoan parasite *Plasmodium falciparum* is responsible for more than 500 million clinical cases of tropical malaria annually. Although exposed to high fluxes of reactive oxygen species, *Plasmodium* lacks the antioxidant enzymes catalase and glutathione peroxidase. Thus, the parasite depends on the antioxidant capacity of its host cell and its own peroxidases. These are fuelled by the thioredoxin system and are considered to represent the major defense line against peroxides. Five peroxidases that act in different compartments have been described in *P. falciparum*. They include two typical 2-Cys peroxiredoxins (Prx), a 1-Cys Prx, the so-called antioxidant protein (AOP), which is a further Prx acting on the basis of a 1-Cys mechanism, and a glutathione peroxidase–like thioredoxin peroxidase. Because of their central function in redox regulation and antioxidant defense, some of these proteins might represent highly interesting targets for structure-based drug development. In this article we summarize the present knowledge on the thioredoxin and peroxiredoxin metabolism in malaria parasitized red blood cells. We furthermore report novel data on the biochemical and kinetic characterization of different thioredoxins, of AOP, and of the classic 1-Cys peroxiredoxin of P. falciparum. *Antioxid. Redox Signal.* 8, 1227–1239.

REDOX NETWORKS IN NONINFECTED RED BLOOD CELLS

As the main function of the red blood cell (RBC) is the transport of molecular oxygen and carbon dioxide, it is constantly exposed to potential sources of reactive oxygen species (ROS) and reactive nitrogen species (RNS). RBCs therefore need to rely on an effective antioxidative system to protect enzymes, lipids, and other biomolecules from oxidative damage. Circulating red cells furthermore represent mobile free radical scavengers for the whole organism (74). To serve these needs, the RBC is well equipped with nonenzymatic [*e.g.*, glutathione (GSH), ascorbic acid, vitamin E] and enzymatic antioxidants (8, 9, 74). Most of the enzymatic antioxidant capacity of whole blood is actually localized in the erythrocytes. Compared with other cell types, RBCs display high activities of superoxide dismutase (SOD), thioredoxin peroxidase 1 (PrxII, TPx1), glutathione peroxidase (GPx), and glutathione reductase (GR), reflecting the need of RBCs for an efficient detoxification machinery (74).

The glutathione system in erythrocytes

Because mature red cells lack mitochondria for oxidative phosphorylation, they generate adenosine triphosphate (ATP) through glycolysis. Besides ATP, the erythrocyte produces NADPH in the pentose phosphate pathway (PPP) and NADH for the reduction of continuously produced methemoglobin (MetHb). Part of the NADPH is used for the reduction of MetHb by NADH- and NADPH-dependent MetHb reductase (cytochrome *b*5 reductase) and, most importantly, for the reduction of the erythrocyte glutathione pool. The antioxidant glutathione is the metabolically most important thiol, with cellular concentrations ranging between 2 and 10 m*M* (79). GSH plays a pivotal role in the antioxidant defense of cells through the maintenance of the redox state of protein–SH moieties, the reduction of the hy-

Interdisciplinary Research Center, Justus Liebig University, Giessen, Germany.

drogen and lipid peroxides, and the extrusion of toxic compounds. It serves as a detoxifying or antioxidant substrate for enzymes such as glutathione transferases (GSTs) and the selenium-containing GPx. Oxidized glutathione (GSSG) is reduced to GSH by NADPH through the flavoenzyme glutathione reductase (19, 76; for reviews, 22). A further component of the proteinaceous redox system of RBCs is glutaredoxin (Grx), which is a specific glutathionyl disulfide oxidoreductase (47, 60). Monothial glutaredoxins are able to reduce specifically mixed disulfides with glutathione via a monothiol mechanism in which only an N-terminal low-pK_a Cys residue is required. Glutaredoxins catalyze glutathionylation reactions (formation of mixed disulfides), which is an important redox regulatory mechanism in sensing cellular redox potential in mammalian cells under oxidative stress (23).

The thioredoxin system in erythrocytes

Besides GSH, the second important redox regulator in RBCs is thioredoxin (Trx). Thioredoxins are members of an evolutionarily conserved family of redox-active proteins containing a conserved active-site dithiol motif ($WC^{32}GPC^{35}K$ in hTrx1) with molecular masses of ~12 kDa. Additionally, human Trx1 contains three other cysteine residues, Cys62, 69, and 73. Of these, Cys73 is involved in Trx dimerization (82). Thioredoxins are present at micromolar or submicromolar concentrations and play a major role in reducing protein disulfides by using their specific dithiol/disulfide active site (2). Figure 1 summarizes the basic reaction catalyzed by the thioredoxin system. Trx is reduced from its inactive form containing a disulfide (S-S) bond to the active dithiol form by NADPH through the selenoflavoenzyme thioredoxin reductase (TrxR). In brief, the oxidized TrxR binds NADPH and then transfers the electrons to its active site where the disulfides are reduced. Then the C-terminally located Cys-Sec takes over the electrons and moves into a more solvent-accessible position where Trx and other substrates are reduced (3, 5, 46, 83). The dithiol form of Trx in turn is reoxidized while providing reducing equivalents to target molecules. Through this reaction, Trx serves as electron donor for enzymes such as ribonucleotide reductases, Trx-dependent peroxidases, ASK1 (involved in apoptotic regulation), methionine sulfoxide reductases, and various transcription factors (51). Moreover, Trx has been assigned a role in various cellular functions, including antioxidant defense, redox-sensitive signal transduction, transcriptional activation of stress-response genes, ribonucleotide reduction in synthesis of deoxyribonucleotides for DNA repair, postinjury cell proliferation, tumor resistance to anticancer drugs, and apoptosis (81).

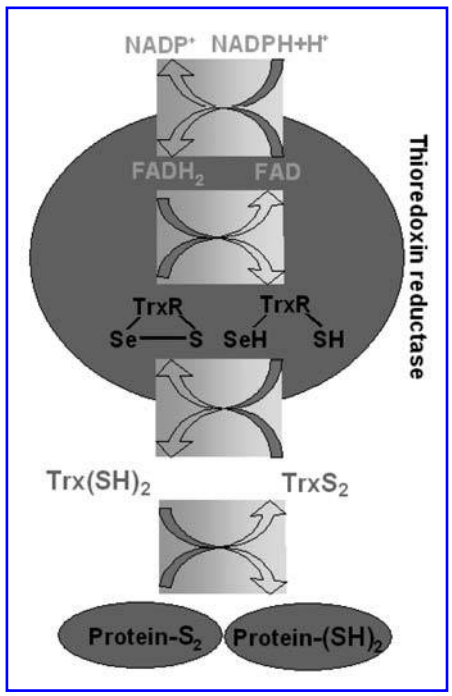

FIG. 1. General scheme of the thioredoxin reductase reaction. In human TrxR, the C-terminal redox center that is shown in this diagram is represented by a Sec-Cys pair (as indicated), in *Plasmodium,* it is represented by a Cys-Cys pair.

Erythrocytes contain a functional Trx system comprising Trx reductase, Trx (11, 31, 45), and at least three peroxiredoxins. The Trx-dependent peroxiredoxin 1 (PrxI, TPx2) is highly expressed in erythrocytes, believed to play a protective role against ROS–mediated damage, and was found to bind to integral membrane proteins or cell membranes via its C-terminal region (12). Furthermore, the Trx-dependent peroxiredoxin 2 (PrxII, TPx1) has been shown to exist in red blood cells (44), as well as a 1-Cys Prx (PrxVI, AOP2), which acts via a 1-Cys mechanism (see 30, 85 for review). AOP2 has been suggested to prefer GSH as reducing substrate and to require glutathionylation for activation (45, 77).

The major source for ROS in the RBC is the autoxidation of oxyhemoglobin, leading to the production of the superoxide anion, which dismutates to hydrogen peroxide (48). The main antioxidant defense lines in RBCs were thought to be predominantly the superoxide dismutase, the heme-dependent catalase, and the selenoprotein glutathione peroxidase as enzyme systems. Glutathione is NADPH-dependently regenerated and serves as indirect and direct ROS scavenger (16, 26, 28, 53, 55). Nagababu *et al.* (53) suggested that the unique role for GPx might be the ability to react with hydrogen peroxide "generated in the close proximity to the RBC membrane in conjunction with the autoxidation of membrane bound hemoglobin." Recently the importance of the Trx system in detoxification of ROS in RBCs was demonstrated as loss of the peroxiredoxin 1 (PrxI; thioredoxin peroxidase 2, TPx2) causes severe fatal hemolytic anemia in aging mice (56). Another study by Lee *et al.* (43) showed that mice deficient in peroxiredoxin 2 (PrxII; thioredoxin peroxidase 1, TPx1) contained markedly higher levels of ROS in dense RBC fractions and developed nonfatal hemolytic anemia, which also pinpoints the pivotal role for Trx-dependent mechanisms in ROS defense of RBCs (43). Some general aspects of these antioxidant players as well as details concerning the *Plasmodium falciparum* Trx network are presented below.

REDOX NETWORKS IN INFECTED RED BLOOD CELLS

A close connection between the host redox system and *Plasmodium falciparum*, the causative agent of tropical malaria, which multiplies in RBCs, has been demonstrated by *experimenta naturae*. Host glucose 6-phosphate dehydrogenase deficiency as well as various hemoglobinopathies like sickle cell anemia and thalassemias, which have been shown to affect the redox equilibrium of the host RBC, confer a selective advantage by reducing mortality of human malaria. The detoxification of ROS and RNS is a challenge for erythrocytes infected with *Plasmodium*. Large quantities of toxic redox-active products are generated as a result of the high metabolic rate of the rapidly growing and multiplying parasite, the host immune response to the infection, and the degradation of hemoglobin by the parasite, which leads to the release of the free toxic heme (see 9 for review). However, the parasite possesses enzymes for glutathione biosynthesis and a whole range of enzymatic antioxidant defense mechanisms (8). Furthermore, it supplies antioxidant moieties like GSH to the host (4).

A complete glutathione system comprising NADPH, highly active glutathione reductase, glutathione, Grx, and different Grx-like proteins as well as a functional glutathione-dependent glyoxalase system and a glutathione *S*-transferase with peroxidase activity have been described for *Plasmodium* (see 7–9 for reviews). In addition, a functional Trx system (see later), two functional superoxide dismutases (20, 75), as well as two lipoamide dehydrogenase–like proteins, which belong to the same disulfide reductase family as GR and TrxR, have been identified (54). A stunning feature of the malarial parasite's redox network is the absence of two genes that code for enzymes involved in ROS detoxification in non-infected erythrocytes (see earlier): catalase (detoxifying hydrogen peroxide) and glutathione peroxidases (glutathione-dependently reducing hydroperoxides). Like its host, the malarial parasite possesses a functional Trx system intensively involved in detoxification of ROS. It comprises NADPH, Trx reductase (PfTrxR), three thioredoxins (PfTrx1, PfTrx2, and PfTrx3), two Trx-like proteins (PfTlp1 and PfTlp2), Trx-dependent peroxidases (PfTPx) and plasmoredoxin (Plrx), a 22-kDa Trx-like protein unique for malarial parasites (see data presented later; 7, 54, 63, 65, 66 for reviews, 58). Figure 2 gives an overview of the redox networks in malarial parasites. In the following sections, we summarize the present knowledge on Trx, Trx reductases, and peroxide-detoxifying enzymes in malarial parasites. A literature review is complemented with recently obtained data.

Methods for recombinant protein production and biochemical analyses

Cloning and overexpression of Trx, Tlp, TrxR2, Pf1-Cys-Prx, and AOP. Thioredoxins and Trx-like proteins (Tlp) as well as AOP (72) and TrxR2 of *Plasmodium falciparum* described here were identified by BLASTs of the database PlasmoDB (http://www.plasmoDB.org) and NCBI (http://www.ncbi.nlm.nih.gov/BLAST/). If the respective recombinant thioredoxins could be reduced by PfTrxR, we named them Trx (Trx1–3); if this reduction could not be shown, we named the proteins Trx-like protein (Tlp1 or Tlp2). All seven genes were polymerase chain reaction (PCR) amplified from a gametocyte *P. falciparum* cDNA library with perfect-match primers (MWG-Biotech), as described for PfTrx1 (34) with slight experimental variations. The gene encoding C-terminally LE(H_6)-tagged Pf1-Cys-Prx was directly cloned into pET28 (Novagen) and expressed in *Escherichia coli* BL21 cells. All other respective PCR products were cloned (Ready-to-go-T_4-DNA-Ligation kit, Amersham) into a vector for sequencing (usually pBluescriptSK$^+$, Stratagene) and then into an expression vector (usually pQE30, Qiagen, which adds an N-terminal hexahistidyl-tag to the recombinant protein). After heterologous overexpression in *E. coli* cells (strain M15, XL1Blue, BL21, C41, or C43), the proteins were purified over an Ni-NTA affinity matrix (Qiagen) according to the manufac-

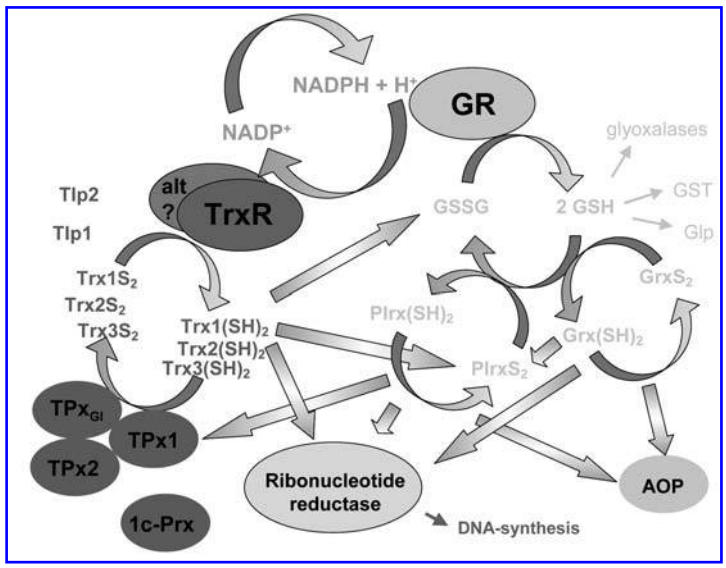

FIG. 2. The redox networks in *P. falciparum*. The glutathione system and the thioredoxin system are operating in malarial parasites in parallel. Both systems are NADPH dependent. The thioredoxin system fuels four peroxidases. The glutathione system is—via glutaredoxin—involved in the reduction of AOP. Plasmoredoxin represents a link between the two systems.

turer's recommendations. The purity of the proteins was proven by sodium dodecylsulfate (SDS) gel electrophoresis. In the case of Pf1-Cys-Prx, all enzymatic assays, the molecular modeling, as well as the gel filtration analyses were carried out as described for the peroxiredoxin TgPrx2 from *Toxoplasma gondii* (17). For high-performance liquid chromatography (HPLC)/electrospray ionization (ESI)-mass spectrometry (MS) analysis, freshly purified Pf1-Cys-Prx (0.7 m*M*) was diluted 1:100 in 300 m*M* NaCl, 50 m*M* $Na_xH_yPO_4$, pH 7.4, and incubated with or without 5 m*M* GSH or 5 m*M* GSSG for 6 h at room temperature.

Insulin assay. Reductive activity of the purified Trx and Tlp proteins was assessed by measuring the reduction of insulin disulfide bonds, which increases turbidity of the solution (A_{600}) (64). From 5 to 20 μ*M* of the respective protein is mixed with 50 m*M* Tris HCl, 2 m*M* ethylenediaminetetraacetic acid (EDTA), pH 7.4, 0.17 m*M* insulin, and the assay is started with 1 m*M* dithiothreitol (DTT), which reduces a redox active protein, but not the peptide hormone in an appropriate time. The negative control omitting the redox protein is carried out simultaneously.

Thioredoxin activity. Reduction by Trx reductase was used to determine the Trx activity of the purified Trx and Tlp proteins. Therefore 100 μ*M* NADPH and different Trx/Tlp concentrations were mixed in 100 m*M* sodium phosphate, 2 m*M* EDTA, pH 7.4, in 0.5 ml volume. After starting the assay with ~10 n*M* PfTrxR, consumption of NADPH was measured as absorption decrease at 340 nm. PfTrxR and PfTrx were prepared as described by Kanzok *et al.* (34).

PfGR was expressed and purified according to Farber *et al.* (22) and kindly provided by Prof. Schirmer, Biochemiezentrum, Heidelberg, Germany. PfGrx was obtained according to Rahlfs *et al.* (64). Expression and purification of PfPlrx were carried out as described in Becker *et al.* (6).

Thioredoxin 1

The biochemical properties of Trx1 were reported previously (34). As described later (65), Trx1 is also capable of detoxifying peroxides directly. In a coupled assay system with NADPH, TrxR, Trx and peroxides, second-order rate constants of 6.7 ± 0.7 M/s for the reaction with H_2O_2, 1.3 ± 0.8 M/s for cumene hydroperoxide, and 0.5 ± 0.2 M/s for *tert*-butyl-hydroperoxide were determined at 25°C. Along these lines, we tested further substrates of Trx1 and showed that Trx1 also is able to reduce dehydroascorbate with a k_2 of 59 ± 5 M/s, lipoic acid with a k_2 of 190 ± 20 M/s, and lipoamide with a k_2 of 390 ± 44 M/s at 25°C. When tested in direct comparison, *P. falciparum* glutaredoxin reduces dehydroascorbate with a k_2 of 920 ± 83 M/s, lipoic acid with a k_2 of 550 ± 45 M/s, and lipoamide with a k_2 of 660 ± 50 M/s (57). Because these second-order rate constants are rather low, a physiologic function of the respective reactions has to be questioned.

In addition to Trx1 and plasmoredoxin, the genes of four other proteins with sequence similarities to Trx were identified in the genome of *P. falciparum* (Table 1 and Fig. 3). Recently studied properties of the respective proteins are described later.

Thioredoxin 2

The full-length coding sequence of Trx2 (Trx2l; the PlasmoDB annotation MAL13P1.225 was found to be correct), as well as a variant lacking the putative secretory signal (Trx2k) and a variant lacking the complete putative apicoplast target sequence (Trx2sk) (see Fig. 3) were PCR amplified, cloned, and overexpressed. Up to 60 mg protein was obtained from 1 L cell culture (Trx2sk); however, all variants of the protein-containing target sequences were hardly soluble and prone to precipitation. Extensive tests to improve solubility were not successful. Trx2 is predicted to contain a secretory signal peptide as well as a transit peptide for apicoplast targeting. Whereas programs define the cleavage site of the signal peptide, the length of the whole target signal is not indicated. Thus the possible start of the putative mature protein was deduced from alignments with other Trx proteins (Fig. 3). The respective protein was shown to be reduced by TrxR. However, because of the solubility problems, this reaction was hard to quantify. Direct comparison of 20 μ*M* Trx1 and

TABLE 1. THIOREDOXINS AND THIOREDOXIN-LIKE PROTEINS OF *PLASMODIUM FALCIPARUM*

	Trx1	*Trx2*	*Trx3*	*Tlp1*	*Tlp2*
Chromosome	14	13	9	14	9
Number of exons	2	4	5	1	5
Active site	WCGPC	WCQAC	WCKPC	WCGPC	WCAPC
Molecular mass	11.7	18.6	20.9	14.4	14.8
(- N-term) [kDa]	—	15.7	17.8	—	?
$\epsilon_{280\ ox}$ [mM^{-1}cm^{-1}]	11.7	16.02	22.2	15.25	12.45
Signal peptide	No	Yes	Yes	No	?
Putative function	Cytosolic thioredoxin	Appicoplast thioredoxin	Apicoplast thioredoxin	Dynein regulator	Long 5′-UTR, membrane associated? mitochondrial?
Reduction by PfTrxR	Yes	Yes	Yes	No	?
Reduction of insulin	Yes	Yes	Yes	Yes	Yes
Reduction of GSSG	Yes	Yes	Yes	No	?
Substrate of PfTPx1	Yes	Yes	?	No	?

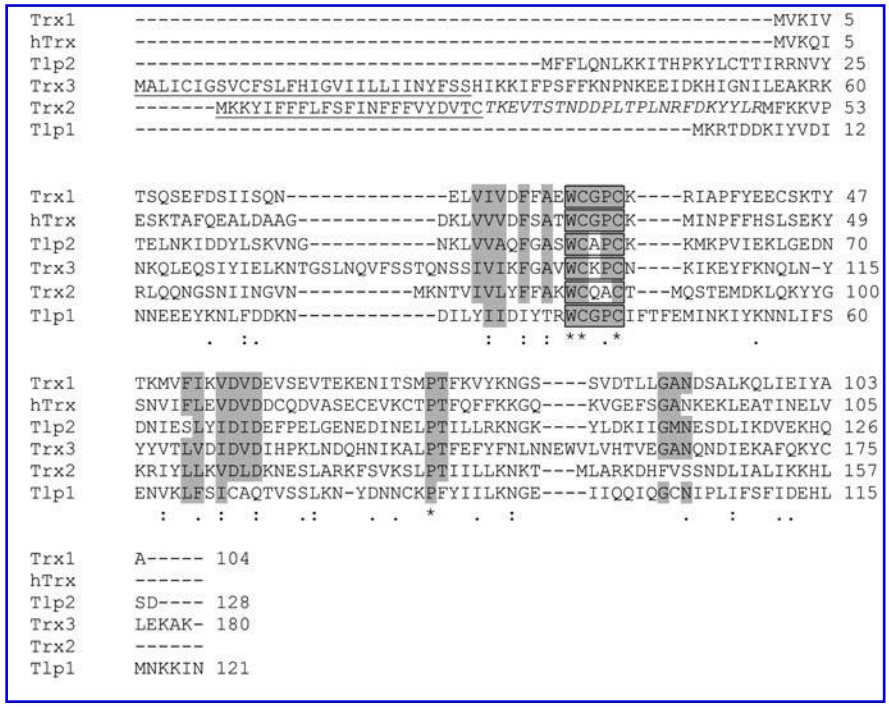

FIG. 3. Multialignment of different thioredoxins and thioredoxin-like proteins of *Plasmodium falciparum*. Human thioredoxin is given for comparison. Trx1 has highest similarities to hTrx; the others deviate in size and remarkably with respect to the conserved active site (*boxed*). For more details, see text and Table 1. Accession numbers: Trx1 (AAF34541), Trx2 (AAQ05974), Trx3 (AAQ76284), Tlp1 (AAQ07982), Tlp2 (AAQ07983), hTrx1 (AAA74596). Putative signal sequences are underlined; the putative apicoplast transit peptide in Trx2 is given in italics.

Trx2sk, in the Trx assay, revealed 40 times higher activities of PfTrxR with Trx1 (16.5 vs. 0.4 U/mg at 25°C). Further experiments showed concentration dependency for Trx2 as electron donor for TPx1 as well as reduction of GSSG by Trx2k and *vice versa*. Although these findings need further confirmation and quantification, they point to a functional similarity between Trx2 and Trx1, with Trx2 located putatively in the apicoplast.

Thioredoxin 3

The PlasmoDB prediction chr9.phat_172 coding for a putative Trx was found to be correct, as proven by experimental methods. The Trx3 sequence lacking the predicted signal peptide (Fig. 3) was chosen for overexpression in various bacterial cell lines, resulting in a maximal yield of 6.5 mg Trx3 per 1 l culture in C43 cells. Although low yields of pure protein did not make detailed biochemical characterization possible until now, Trx3 was found to be redox active in the insulin assay and could be reduced by PfTrxR.

Thioredoxin-like protein 1

The PlasmoDB annotation PF14_0590 was found to be correct for this gene. From one *E. coli* culture, 5–9 mg recombinant Tlp1 was obtained. The protein was found to be rather instable, which could be partially prevented by the addition of bovine serum albumin (BSA; 0.5 mg/ml). The protein active in the insulin assay did, however, not serve as substrate of TrxR (≤30 μ*M* Tlp1 was used in the assay) and was thus named Tlp1.

Tlp1 shows sequence similarities to thioredoxins (Fig. 3) as well as to dyneins; the dynein family comprises large, medium, and light chains, so Tlp1 might represent a small dynein subunit. Dynein light chains (DLCs) were recognized as members of the Trx superfamily by Patel-King *et al*. (61) and have also been described in other parasites (59).

Thioredoxin-like protein 2

The PlasmoDB prediction chr9.phat_260 was found to be correct (Fig. 3). Different approaches were made for heterologous overexpression of Tlp2 in *E. coli* by using different vectors, strains, and expression conditions. In parallel, *in vitro* translation was successfully tested by Dr. Frank Bernhard, Institute of Biophysical Chemistry, Frankfurt. However, the protein was associated with the pellet and could not be purified. Only a small amount of Tlp2 could be obtained by expression in pRSETA by using *E. coli* C41 cells. Identity of the purified protein was confirmed by Western blot using His-tag antibodies. Tlp2 was active in the insulin assay. Trx activity of Tlp2 with TrxR cannot be ruled out, but until proven, the protein is listed as a "Trx-like protein."

Thioredoxin reductases

The Trx reductase of *P. falciparum* is a homodimeric, FAD-dependent oxidoreductase (for reviews see 7, 9, 54, 66). The role of an additional C-terminal redox center in catalysis has been proven (as is the case for mammalian TrxRs), and its interaction with the active site disulfide-dithiol has been studied in detail (83 for review). In contrast to the human TrxR, the PfTrxR is not selenium dependent and has a C-terminal active site motif (CGGGKC) differing from human TrxR (Cys-Sec), which makes the parasitic enzyme a prospective potential drug target. As demonstrated by Krnajski *et al*. (40), PfTrxR is indeed essential for erythrocytic stages of *P. falciparum*, which underlines the importance of the Trx system in malarial parasites.

After the genome sequence of *P. falciparum* had become available (27), *in vitro* analysis of the PfTrxR gene locus in our laboratory revealed a second putative start codon upstream of the published start (accession number CAA60574). This alternative start does not involve an additional intron. This 5′-elongation of PfTrxR was confirmed in our laboratory and could be cloned from a cDNA library. The respective recombinant enzyme PfTrxR2 (accession number AAQ07981) was analyzed and found to be very similar in substrate specificity and kinetic and biochemical behavior to the previously studied shorter variant (52, 57). Interestingly, this new N-terminal sequence was not found to be predicted as a target sequence; thus the location of the two alternative TrxRs is presently studied in more detail. In this context, it is worth mentioning that *Drosophila melanogaster* TrxR also contains an unusual N-terminal elongation, which is not predicted to be a signal sequence, either. For this enzyme, transport into the mitochondrium has been demonstrated experimentally (49). As shown for mitochondrial SOD, for example, target sequences of redox active proteins, which are not yet identified by the available prediction programs, do exist in *Plasmodium* (75).

Thiol-dependent hydroperoxidases in malaria-infected red blood cells

Proteins capable of peroxide degradation include heme-dependent hydroperoxidases (*e.g.*, catalases) and thiol-dependent hydroperoxidases such as glutathione peroxidases and peroxiredoxins. Most of the mammalian glutathione peroxidases possess a selenocysteine residue at the active site, whereas all Prx characterized so far react via a conserved cysteine residue. The overall contribution of Prx to peroxide detoxification depends on the organism/tissue investigated because several of these enzymes possess rather low catalytic efficiencies in the range of 10^5 M/s in comparison with 10^8 M/s determined for GPx. However, the high abundance of Prx, their high intracellular concentrations, as well as the large number of isoforms support their impact (24, 30, 85). Prx have been suggested also to be involved in detoxification of RNS such as peroxynitrite (10, 39, 58, 71, 80), regulation of apoptosis (87), and modulation of cellular differentiation and signal-transduction pathways (30, 32, 33, 42, 85).

Detoxification of hydroperoxides catalyzed by Prx generally involves the oxidation of a conserved "peroxidatic" cysteine residue located in the N-terminal domain of the protein (14, 15, 17, 21, 50, 72). Typical 2-Cys Prx are active as homodimers and possess a second "resolving" cysteine residue located in the C-terminal domain of the second subunit (13, 21, 29, 58). In contrast, atypical 2-Cys Prx form an intramolecular disulfide as reaction intermediate (30, 73, 85). 1-Cys Prx possess only one catalytically active cysteine residue, which is directly reduced by the thiol-containing substrate (30, 32, 85). Interestingly, during recent years, several mechanistic exceptions to this widely accepted alignment-based classification of Prx into 1-Cys Prx, typical 2-Cys Prx, and atypical 2-Cys Prx have been reported (see later). Most of the 1-Cys Prx can be reduced *in vitro* only by nonphysiologic low-molecular-weight thiols, whereas many eukaryotic 2-Cys Prx are reduced by Trx (13, 17, 24, 32, 50, 63, 85). Prx of plants have furthermore been reported to accept glutaredoxin as electron donor (69, 70), and several bacterial Prx have even been shown to be fused with a Grx domain at their C-terminus (68).

Because *Plasmodium* lacks GPx and catalase, peroxiredoxins are likely to be of particular importance for ROS and RNS defense in this organism (8, 54, 66).

Five peroxidases have been described in *P. falciparum.*

1. Trx peroxidase 1 (TPx1) and TPx2 are classified as typical 2-Cys Prx (63, 66). The substrate specificity and physiologic role of TPx2—which is localized in the mitochondria and is expressed during trophozoite and schizont stages (86)—remains to be elucidated (63). In contrast, the contribution of the cytosolic, Trx-dependent TPx1 (1, 36, 41, 58, 63) to peroxide degradation *in vivo* has been demonstrated by knockout studies: in comparison with wild-type parasites, erythrocytic stages of PfTPx1 knockout parasites exhibit a reduced growth rate when treated with paraquat and sodium nitroprusside, demonstrating that the enzyme is involved in protecting the parasite against oxidative and nitrosative stress (39). In the absence of these compounds, however, knockout parasites grow normally, suggesting that TPx1 is not essential for the detoxification of endogenously produced ROS or RNS or both in *Plasmodium* cultures. Protein and mRNA levels of TPx1—which is constitutively expressed in the parasite cytoplasm throughout the erythrocytic stages (37, 86)—are increased in response to exogenous oxidative stress (1). The protein oligomerizes to $(\alpha_2)_5$ decamers and reacts very efficiently with H_2O_2 (k_{cat}/K_m ~6.7×10^6 M/s at 30°C) (1, 84, see 85 for review on oligomerization). Furthermore, TPx1 possesses peroxynitrite reductase activity (4 x 10^5 M/s at pH 7.4 and 25°C), and accepts electrons from Plrx (58).
2. The third *P. falciparum* peroxidase Pf1-Cys-Prx (38, 41, 66, see also later) is classified as a 1-Cys Prx and shows elevated expression during the trophozoite and schizont stage (37, 86). Recently it was suggested that an interaction between cytosolic Pf1-Cys-Prx and ferriprotoporphyrin IX protects *P. falciparum* from oxidative stress (35).
3. The fourth *P. falciparum* Prx-named antioxidant protein (AOP, see also later), was identified, and its crystal structure has been solved (72).
4. The fifth peroxidase is not a classic Prx but shows high sequence similarity with glutathione peroxidases, although it prefers Trx as reducing substrate (78).

Antioxidant protein: a novel peroxiredoxin of P. falciparum

A *P. falciparum* protein with high sequence similarity to human peroxiredoxin V as well as to certain plant and bacterial peroxidases (62, 68, 70, 73) was identified in PlasmoDB and designated PfAOP. A homologue of PfAOP was found in *P. yoelii* and in *Toxoplasma gondii*. Microarray data available on PlasmoDB (acc no. MAL7P1.159) show that the AOP gene is transcribed throughout the blood-stage forms of *P. fal-*

ciparum, reaching maximal transcription in the trophozoite stage. This transcription profile is paralleled by the expression of glutaredoxin, the reductant of AOP identified in our study (see below).

Because signal peptide predictions by TargetP and SignalP resulted in high probabilities (85% and 92%, respectively) for a target sequence in AOP, a clone coding for the protein without the 24 N-terminal amino acid residues, which corresponds to the putative signal, was generated. Although expression was successful, this protein species showed no peroxidase activity. The target-prediction program of PlasmoDB (five of five parameters are fulfilled) as well as PATS (score of 0.836) indicated an apicoplast localization of AOP. Because transit peptide cleaving-site predictions are not reliable so far, a sequence for the putative mature protein was derived from aligning AOP with other known peroxidase sequences. A corresponding PCR product comprising 546 bp was generated and inserted into pQE30. PfAOP was overexpressed in *E. coli* M15 cells; up to 40 mg/L protein was obtained from 1 L cell culture.

Antioxidant protein of P. falciparum acts on the basis of a 1-Cys mechanism

As reported recently (72), we crystallized AOP, and its three-dimensional structure at 1.8 Å indicates that PfAOP belongs mechanistically to the family of 1-Cys Prx. To prove this hypothesis, we assessed the reduction state of recombinantly produced AOP and the accessibility of cysteine residues experimentally (Table 2). The 20 μ*M* AOP was incubated for 20 min at 25°C in the dark in 100 m*M* Tris, 1 m*M* EDTA, pH 8.0, in the presence and absence of denaturing and/or oxidizing agents. Then 200 μ*M* DTNB was added. After 20-min incubation, the concentration of produced TNB^- was measured at 412 nm (ϵ = 13.6 m*M*/cm). To account for cysteine residues that were reduced but not accessible in the native state, measurements were also done in the presence of 1% SDS and 6 *M* GuHCl, respectively. Furthermore, we tested whether a change of the reduction state of AOP occurred in the presence of 200 μ*M* H_2O_2. For each sample, an AOP-free control was carried out. Under the experimental conditions chosen and given the fact that AOP contains two cysteine residues, completely reduced PfAOP should result in 40 μ*M* sulfhydryl groups. As summarized in Table 2, the native protein possesses one reduced *and* accessible cysteine residue after purification under nonreducing conditions. The presence of 1% SDS (69) did not significantly change this result; however, with 6 *M* GuHCl, the second cysteine became accessible and was also found to be reduced. The addition of peroxide substrate (H_2O_2 or tBOOH) to the incubation mixture led to a complete oxidation of SH groups. Thus both cysteine residues of the protein are present in reduced form, even when AOP is prepared in the absence of a reductant. This observation supports the 1-Cys mechanism of PfAOP and is in accordance with data obtained for poplar Prx. However, whereas both sulfhydryl groups were detectable in the native poplar protein (70), the second cysteine residue of PfAOP became accessible only after treatment with 6 *M* GuHCl. This result is clearly reflected by the x-ray structure of PfAOP, which indicates that Cys59 is the peroxidatic cysteine, and Cys85 is buried as part of a well-defined core β-sheet, which is difficult to access (72).

Table 2. Accessibility of PfAOP SH Groups

		[SH-groups] (μM)
AOP without denaturants		16.1 ± 2.0
With 1% SDS	Without peroxide	18.4 ± 2.5
	With 200 μ*M* H_2O_2	1.7 ± 0.3
	With 200 μ*M* tBOOH	1.5 ± 0.4
With 6 *M* GuHCl	Without peroxide	34.6 ± 4.2
	With 200 μ*M* H_2O_2	1.0 ± 0.2

20 μ*M* AOP was incubated in 100 m*M* Tris, 1 m*M* EDTA, pH 8.0, with or without oxidants/denaturating agents for 20 min. 200 μ*M* DTNB was added, and after a second incubation period, the formation of TNB anions was measured at 412 nm, and the concentration of SH-groups was calculated. Data represent mean values of three independent determinations.

As shown by SDS-PAGE under oxidizing conditions, PfAOP can form higher aggregates as well as a second prominent monomer band with a seemingly lower molecular mass than AOP (~20 kDa instead of 25 kDa). Such double bands have been reported for other peroxiredoxins including PfTPx1 (63) and AOP from *Populus tremula* (PtAOP) (70). Purification in the presence of DTT could prevent the doublet formation in PfTPx1, which suggests that the double band does represent an oxidation product. This also is the case for PfAOP. The double band appears only under denaturing nonreducing conditions. Gel-filtration studies on PfAOP and structural data obtained from x-ray studies show that PfAOP is present as a non-covalently linked dimer (72).

AOP reduces peroxides in the presence of glutaredoxin

In the presence of 150 μ*M* NADPH, 0.5 U/ml PfGR, 1 m*M* GSH, 100 μ*M* tBOOH, and glutaredoxin, a peroxidase activity correlating directly with the AOP concentration (0–40 μ*M*) was determined. This reaction was also clearly dependent on the Grx concentration (0–20 μ*M*). Very similar results, although with lower activities, were determined for thioredoxin (0–20 μ*M*) (57). However, in both cases, high AOP concentrations were required to obtain good signals. Thus the order of the reaction will have to be studied in further detail, and the influence of glutaredoxin and the peroxide on the stability of AOP will have to be assessed. Preferred hydroperoxides reduced by AOP in this reaction were tBOOH and phosphatidyl hydroperoxide, whereas H_2O_2 and CuOOH led to less NADPH consumption.

Based on these preliminary data, AOP is the first Grx-dependent peroxiredoxin described in *P. falciparum* and the first peroxiredoxin turning over lipid hydroperoxides. In addition, it is the first Grx-dependent AOP reported in nonplant organisms. This aspect is of particular importance because *P. falciparum* lacks a genuine GPx, and peroxidase activity was so far thought to depend on the thioredoxin system in this organism. Furthermore, PfAOP might represent a novel member of

the very few Grx-dependent peroxiredoxins described so far. Grx-dependent Prx have previously been assigned to the PrxV class/atypical Prx (30, 85), type C, or type II Prx (68, 70).

Plasmoredoxin reduces AOP

In a further experiment, we studied the reduction of AOP by Plrx on the basis of the reaction of sulfhydryl groups with Ellman's reagent (DTNB). Plrx, a *Plasmodium*-specific redox-active protein of 22 kDa, which belongs to the thioredoxin superfamily (6), was found to be an electron donor for PfAOP (Table 2). Plrx is a substrate neither for PfGR nor for PfTrxR but can be reduced by Grx and provides electrons to ribonucleotide reductase.

After removal of excess DTT, prereduced Plrx (100 μ*M*) was mixed with 40 μ*M* AOP in the presence/absence of 200 μ*M* tBOOH. Controls were carried out with Plrx alone to assure its complete reduction. Other controls contained Plrx and peroxide to account for the peroxidase activity of plasmoredoxin (see Table 3). Determination of SH-groups was carried out immediately after mixing as well as 15 min after incubation. For that purpose, an aliquot of each sample was diluted by a factor of 10 with 100 m*M* potassium phosphate, 2 m*M* EDTA, pH 8.0, to reach final concentrations of 10 μ*M* Plrx, 4 μ*M* AOP, and 20 μ*M* tBOOH. Absorption at 412 nm was measured and taken as blank. After addition of 400 μ*M* DTNB, absorption was determined, and the concentration of SH-groups was calculated. To include a positive control, the whole experiment was carried out in parallel by using PfTrx instead of Plrx. As shown in Table 3, in the presence of AOP and peroxide, approximately one reducing equivalent per Plrx molecule was immediately transferred to AOP (and from there to tBOOH), again supporting the 1-Cys mechanism of AOP. After 15 min, a second SH-group was oxidized, which might be due to direct oxidation of Plrx or to further transfer of electrons from Plrx to AOP.

The reduction of AOP by Plrx represents a novel redox reaction and is unique for malarial parasites. As described earlier, apart from Trx1, Grx1, and Plrx, four other thioredoxin-like proteins have been detected in the genome of *Plasmodium falciparum* (67). Two of them, Trx2 and Trx3, carry a signal peptide and might also function as reductants for AOP *in vivo*.

Plasmodium falciparum 1-Cys peroxiredoxin Pf1-Cys-Prx

The gene encoding Pf1-Cys-Prx was independently cloned by Kawazu *et al.* in 2000 (38) and Krnajski *et al.* (41) in 2001 by using cDNA from different parasite strains (FCR-3 and 3D7, respectively). Both groups investigated the corresponding recombinant N-terminally His-tagged proteins, which slightly differ in size and amino acid sequence: Kawazu *et al.* demonstrated general anti-oxidative properties (35, 38) in addition to a slight glutathione-dependent peroxidase activity with H_2O_2 (38). In contrast, Krnajski *et al.* (41) qualitatively described a thioredoxin-dependent peroxidase activity with H_2O_2 but not with alkyl hydroperoxides.

To shed some light on this topic, we also cloned and studied Pf1-Cys-Prx. By using a copy of the same 3D7 cDNA library (41), we were able to reproduce the cloning of the gene sequence reported for the strain FCR-3 (38). Thus the amino acid sequence deduced from the PlasmoDB annotation PF08_0131 is confirmed for both *P. falciparum* strains 3D7 and FCR-3. Furthermore, the primer used by Krnajski *et al.* (41) does not encode the C-terminal amino acid sequence QSSL as shown in an alignment but encodes the C-terminal sequence PKRHI.

As presented here, we compared our recombinant C-terminally LE(H_6)-tagged Pf1-Cys-Prx (≤40 mg pure Pf1-Cys-Prx were obtained from 1 L *E. coli* culture) with the highly similar C-terminally LE(H_6)-tagged protein TgPrx2 from *Toxoplasma gondii* (17) by using enzymatic assays, gel-filtration chromatography, mass spectrometry, and molecular modeling.

The physiologic reducing agent of Pf1-Cys-Prx remains unknown

We were not able to detect a significant glutathione-dependent activity with H_2O_2 or *tert*-butyl hydroperoxide. However, incubation of 7 μ*M* freshly purified Pf1-Cys-Prx in the presence of 5 m*M* GSH or 5 m*M* GSSG for 6 h and subsequent analysis by HPLC/ESI-MS revealed that one of the eight cysteine residues per subunit can be glutathionylated. The calculated theoretic mass of unmodified recombinant Pf1-Cys-Prx with or without Met1 is 26.229 or 26.098 kDa, respectively. Furthermore, the measured mass difference of 307 Da between glutathionylated (26.401 kDa) and unmodi-

TABLE 3. REDUCTION OF PFAOP BY PLASMOREDOXIN

	10 μM plasmoredoxin				*10 μM thioredoxin*			
AOP	—	+	+	—	—	+	+	—
tBOOH	—	+	—	+	—	+	—	+
SH-equivalents (μ*M*) t = 10 sec	38.3	26.4	38.1	37.1	20.5	11.8	21.0	20.1
SH-equivalents (μ*M*) t = 15 sec	37.3	21.1	36.8	33.3	20.5	9.1	21.5	18.4

40 μ*M* AOP and 200 μ*M* tBOOH were incubated with 100 μ*M* prereduced Plrx and Trx, respectively, in 100 m*M* potassium phosphate, 2 m*M* EDTA, pH 8.0. After 10-fold dilution in the same buffer, available sulfhydryl groups were detected by addition of 400 μ*M* DTNB. Assays were carried out after 10 sec and after an incubation period of 15 min. Absorption values were corrected for the mean control values without plasmoredoxin. Data represent means of three independent determinations, which differed <10%.

fied (26.094 kDa) protein suggests that the peroxidatic Cys47 of freshly purified Pf1-Cys-Prx was not oxidized to a cysteine-sulfenic or sulfinic acid (Cys-OH or Cys-SO_2H, respectively).

It has been suggested that the 1-Cys Prx from man (PrxVI) is active with GSH only in the presence of a pi type glutathione *S*-transferase (45). First experiments with Pf1-Cys-Prx in presence of the one and only *P. falciparum* glutathione *S*-transferase (18, 25) do not confirm this hypothesis for Pf1-Cys-Prx.

Freshly purified Pf1-Cys-Prx possesses a slight activity with Trx1 and Grx from *P. falciparum*: the specific activity determined with 5.4 μ*M* Trx1 and 0.19 m*M* H_2O_2 was 0.53 U/μmol (20 U/g), and the specific activity with 12 μ*M* Trx1 and 0.15 m*M* *tert*-butyl hydroperoxide was 0.15 U/μmol (5.7 U/g). With 2 m*M* GSH, 10 μ*M* Grx, and 0.15 m*M* or 0.73 m*M* *tert*-butyl hydroperoxide, we measured a specific peroxidase activity of 0.33 U/μmol (13 U/g) or 0.5 U/μmol (19 U/g), respectively. Because the turnover is very low with either Trx1 or Grx, these results do not justify the classification of Pf1-Cys-Prx as a Trx- or Grx-dependent peroxidase, and the physiologic substrates remain unknown. Similar results were obtained for TgPrx2 and several other 1-Cys-Prx (17).

Pf1-Cys-Prx and TgPrx2 show different oligomerization behaviors

Models of dimeric Pf1-Cys-Prx were generated based on the crystal structure of human PrxVI (Fig. 4). Force-field energies and structures of the models are highly similar to the models of TgPrx2 (17), which contains two domains: the N-terminal domain possesses a classic thioredoxin fold, and the C-terminal domain is involved in dimerization. Four of five cysteine residues of TgPrx2 are conserved in Pf1-Cys-Prx (Cys47 at the active site, Cys66, Cys73, and Cys128). Residue Cys209—which is involved in covalent dimerization of TgPrx2—is missing in Pf1-Cys-Prx, whereas Cys101, Cys130, Cys184, and Cys185 of Pf1-Cys-Prx are not conserved in TgPrx2. In contrast to TgPrx2, we were not able to generate a model with an intermolecular disulfide bond by using Cys184′ or Cys185′ of the C-terminal domain as resolving cysteine residue. Furthermore, the oligomerization behaviour of Pf1-Cys-Prx and TgPrx2 *in vitro* is different: in contrast to TgPrx2—which tends to form covalently linked dimers as well as non-covalently linked tetramers and hexamers (17)—aged Pf1-Cys-Prx does not form significant amounts of higher aggregates but tends to monomerize (Fig. 5). Thus despite 47% identical amino acid residues, Pf1-Cys-Prx possesses characteristic features of 1-Cys Prx, whereas TgPrx2 shows similarities to typical 2-Cys Prx. Both proteins are rich in (conserved) cysteine residues. Further studies will show whether these residues possess a common function.

CONCLUSIONS

Based on the fact that the malarial parasite *Plasmodium falciparum* possesses neither a catalase nor a classic GPx, the redox networks of this organism have some unique characteristics. Four peroxiredoxins—TPx1, TPx2, 1-Cys-Prx, and AOP—operate in parallel and in different compartments. In addition, a GPx-like thioredoxin peroxidase is present. Although first data and predictions are available, localization and interacting partners of these enzymes remain to be studied in further detail. AOP, which is likely to be located in the apicoplast, is closely related to respective plant enzymes and might point to the green origin of *Plasmodium*. Plasmoredoxin, as a redox-active protein unique for malarial parasites, connects the glutathione and the thioredoxin systems. Apart

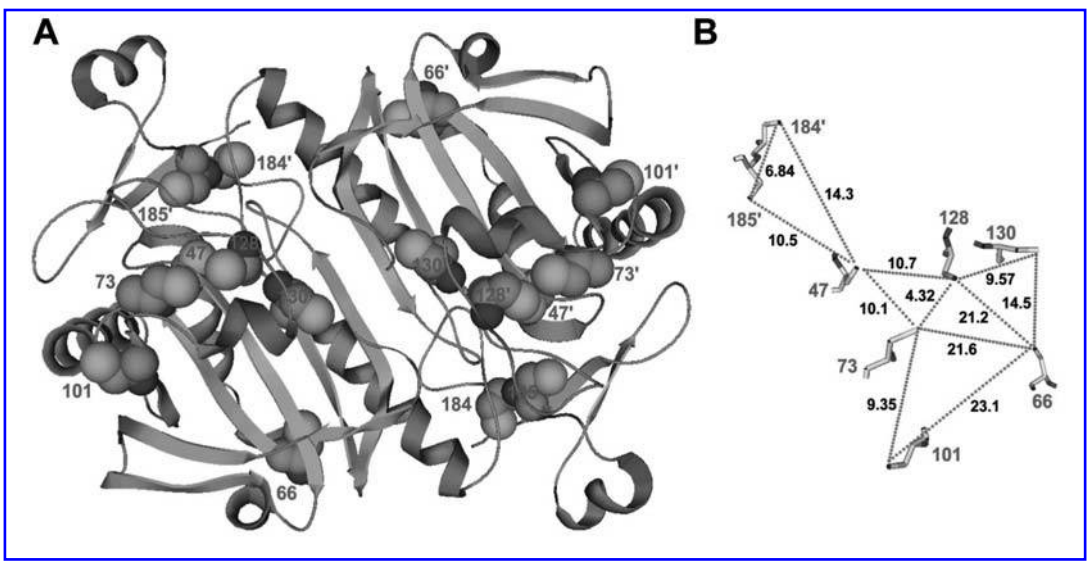

FIG. 4. Molecular model of dimeric Pf1-Cys-Prx based on the crystal structure of human PrxVI (15). **A:** Dimeric Pf1-Cys-Prx possesses a twofold symmetry axis, a central β-sheet, and 16 cysteine residues per homodimer. The force-field energy of the model is –12.1 MJ/mol. **B:** Theoretical distances between cysteine residues are given in angstroms (Å).

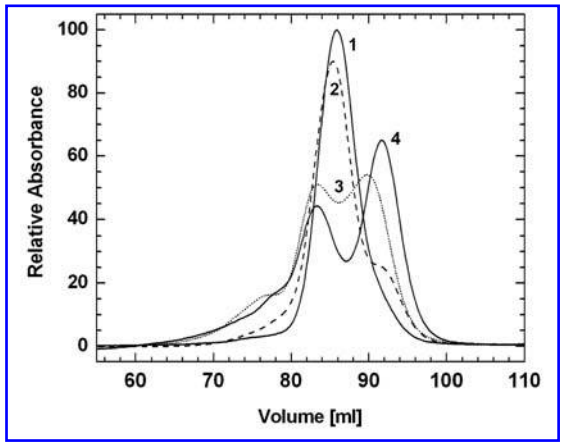

FIG. 5. Pf1-Cys-Prx and TgPrx2 possess a different oligomerization behaviour. Aging of Pf1-Cys-Prx leads to an increase of monomeric protein. Purified recombinant Pf1-Cys-Prx was analyzed under native conditions by gel-filtration chromatography using 50 m*M* $Na_xH_yPO_4$, 300 m*M* NaCl, pH 7.4, as running buffer (compare with data on TgPrx2 in Ref. 17). The protein possesses a calculated molecular mass of 26 kDa per monomer. Freshly purified Pf1-Cys-Prx exists in a rapid monomer–dimer equilibrium (similar to TgPrx2) and elutes in one peak with an apparent molecular mass of ~46 kDa (chromatogram 1). Preincubation of Pf1-Cys-Prx at 25°C or 37°C as well as changing the pH (5.5–8.5) does not significantly influence oligomerization. However, aging or incubation with H_2O_2 leads to the separation of dimeric (M_{app} ~56 kDa) and monomeric (M_{app} ~28 kDa) protein in addition to an increase of monomeric Pf1-Cys-Prx (chromatograms 2–4). Depending on the experiment, 65% monomeric protein was reached in 5–23 days. The appearance of shoulders as well as the aging-related conversion of the chromatogram is (partially) reversible after incubation with 2 m*M* DTT.

from the enzymes encoded by plasmodial genes, the uptake of host cell enzymes, like catalase and peroxidases, which might be used for enhancing the antioxidant defense of malarial parasites, deserves to be studied in further detail (7, 9).

ACKNOWLEDGMENTS

We thank Marina Fischer and Elisabeth Fischer for their excellent technical assistance. The study was supported by the Deutsche Forschungsgemeinschaft (SFB 535, project A12 to K.B. and M.D.).

ABBREVIATIONS

AOP, antioxidant protein; BSA, bovine serum albumin; DTNB, Ellman's reagent: 5,5′-dithio-bis-(2-nitrobenzoic acid); DTT, dithiothreitol; FPLC, fast protein liquid chromatography; GPx, glutathione peroxidase; Grx, glutaredoxin; GSH/GSSG, glutathione/glutathione disulfide; GST, glutathione-*S*-transferase; HPLC/ESI-MS, high-performance liquid chromatography/electrospray ionization mass spectrometry; IFA, indirect immunofluorescence assay; MetHb, methemoglobin; Ni-NTA, nickel-nitrilotriacetic acid; Plrx, plasmoredoxin; PPP, pentose phosphate pathway; Prx, peroxiredoxin(s); RBC, red blood cell; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; TPx, thioredoxin peroxidase; Trx, thioredoxin; TrxR, thioredoxin reductase.

REFERENCES

1. Akerman SE and Müller S. 2-Cys peroxiredoxin PfTrx-Px1 is involved in the antioxidant defence of *Plasmodium falciparum*. *Mol Biochem Parasitol* 130: 75–81, 2003.
2. Arner ES and Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. *Eur J Biochem* 267: 6102–6109, 2000.
3. Arscott LD, Gromer S, Schirmer RH, Becker K, and Williams CH Jr. The mechanism of thioredoxin reductase from human placenta is similar to the mechanisms of lipoamide dehydrogenase and glutathione reductase and is distinct from the mechanism of thioredoxin reductase from *Escherichia coli*. *Proc Natl Acad Sci U S A* 94: 3621–3626, 1997.
4. Atamna H and Ginsburg H. The malaria parasite supplies glutathione to its host cell—investigation of glutathione transport and metabolism in human erythrocytes infected with *Plasmodium falciparum*. *Eur J Biochem* 250: 670–679, 1997.
5. Becker K, Gromer S, Schirmer RH, and Muller S. Thioredoxin reductase as a pathophysiological factor and drug target. *Eur J Biochem* 267: 6118–6125, 2000.
6 . Becker K, Kanzok SM, Iozef R, Fischer M, Schirmer RH, and Rahlfs S. Plasmoredoxin: a novel redox-active protein unique for malarial parasites. *Eur J Biochem* 270: 1057–1064, 2003.
7. Becker K, Koncarevic S, and Hunt NH. Oxidative stress and antioxidant defence in malarial parasites. In: Sherman IW, ed. *Molecular Approaches to Malaria.* ASM Press, Washington: ASM Press, *in press*, 2005.
8. Becker K, Rahlfs S, Nickel C, and Schirmer RH. Glutathione: Function and metabolism in the malarial parasite *Plasmodium falciparum*. *Biol Chem* 348: 551–566, 2003.
9. Becker K, Tilley L, Vennerstrom JL, Roberts D, Rogerson S, and Ginsburg H. Oxidative stress in malaria parasite-infected erythrocytes: Host-parasite interactions. *Int J Parasitol* 34: 163–189, 2004.
10. Bryk R, Griffin P, and Nathan C. Peroxynitrite reductase activity of bacterial peroxiredoxins. *Nature* 407: 211–215, 2000.
11. Cha MK and Kim IH. Thioredoxin-linked peroxidase from human red blood cell: Evidence for the existence of thioredoxin and thioredoxin reductase in human red blood cell. *Biochem Biophys Res Commun* 217: 900–907, 1995.
12. Cha MK, Yun CH, and Kim IH. Interaction of human thiol-specific antioxidant protein 1 with erythrocyte plasma membrane. *Biochemistry* 39: 6944–6950, 2000.
13. Chae HZ, Chung SJ, and Rhee SG. Thioredoxin-dependent peroxide reductase from yeast. *J Biol Chem* 269: 27670–27678, 1994.

14. Chae HZ, Kim IH, Kim K, and Rhee SG. Cloning, sequencing, and mutation of thiol-specific antioxidant gene of *Saccharomyces cerevisiae*. *J Biol Chem* 268: 16815–16821, 1993.
15. Choi HJ, Kang SW, Yang CH, Rhee SG, and Ryu SE. Crystal structure of a novel human peroxidase enzyme at 2.0 A resolution. *Nat Struct Biol* 5: 400–406, 1998.
16. Cohen G and Hochstein P. Glutathione peroxidase: The primary agent for the elimination of hydrogen peroxide in erythrocytes. *Biochemistry* 2: 1420–1428, 1963.
17. Deponte M and Becker K. Biochemical characterization of *Toxoplasma gondii* 1-Cys peroxiredoxin 2 with mechanistic similarities to typical 2-Cys Prx. *Mol Biochem Parasitol* 140: 87–96, 2005.
18. Deponte M and Becker K. Glutathione *S*-transferase from malarial parasites: Structural and functional aspects. *Methods Enzymol* in press, 2005.
19. Dickinson DA, Moellering DR, Iles KE, Patel RP, Levonen AL, Wigley A, Darley-Usmar VM, and Forman HJ. Cytoprotection against oxidative stress and the regulation of glutathione synthesis. *Biol Chem* 384: 527–537, 2003.
20. Dive D, Gratepanche S, Yera H, Becuwe P, Daher W, Delplace P, Odberg-Ferragut C, Capron M, and Khalife J. Superoxide dismutase in *Plasmodium*: a current survey. *Redox Rep* 8: 265–267, 2003.
21. Ellis HR and Poole LB. Roles for the two cysteine residues of AhpC in catalysis of peroxide reduction by alkyl hydroperoxide reductase from *Salmonella typhimurium*. *Biochemistry* 36: 13349–13356, 1997.
22. Färber PM, Arscott LD, Williams CH, Becker K, and Schirmer RH. Recombinant *Plasmodium falciparum* glutathione reductase is inhibited by the antimalarial dye methylene blue. *FEBS Lett* 422: 311–314, 1998.
23. Fernandes AP and Holmgren A. Glutaredoxins: glutathione-dependent redox enzymes with functions far beyond a simple thioredoxin backup system. *Antioxid Redox Signal* 6: 63–74, 2004.
24. Flohé L, Jaeger T, Pilawa S, and Sztajer H. Thiol-dependent peroxidases care little about homology-based assignments of function. *Redox Rep* 8: 256–264, 2003.
25. Fritz-Wolf K, Becker A, Rahlfs S, Harwaldt P, Schirmer RH, Kabsch W, and Becker K. X-ray structure of glutathione *S*-transferase from the malarial parasite *Plasmodium falciparum*. *Proc Natl Acad Sci U S A* 100: 13821–13826, 2003.
26. Gaetani GF, Ferraris AM, Rolfo M, Mangerini R, Arena S, and Kirkman HN. Predominant role of catalase in the disposal of hydrogenperoxide within human erythrocytes. *Blood* 87:1595–1599, 1996.
27. Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, Carlton JM, Pain A, Nelson KE, Bowman S, Paulsen IT, James K, Eisen JA, Rutherford K, Salzberg SL, Craig A, Kyes S, Chan MS, Nene V, Shallom SJ, Suh B, Peterson J, Angiuoli S, Pertea M, Allen J, Selengut J, Haft D, Mather MW, Vaidya AB, Martin DM, Fairlamb AH, Fraunholz MJ, Roos DS, Ralph SA, McFadden GI, Cummings LM, Subramanian GM, Mungall C, Venter JC, Carucci DJ, Hoffman SL, Newbold C, Davis RW, Fraser CM, and Barrell B. Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature 419: 498–511, 2002.
28. Giulivi C, Hochstein P, and Davies KJ. Hydrogen peroxide production by red blood cells. *Free Radic Biol Med* 16: 123–129, 1994.
29. Hirotsu S, Abe Y, Okada K, Nagahara N, Hori H, Nishino T, and Hakoshima T. Crystal structure of a multifunctional 2-Cys peroxiredoxin heme-binding protein 23 kDa/proliferation-associated gene product. *Proc Natl Acad Sci U S A* 96: 12333–12338, 1999.
30. Hofmann B, Hecht HJ, and Flohé L. Peroxiredoxins. *Biol Chem* 383: 347–364, 2002.
31. Holmgren A and Luthman M. Tissue distribution and subcellular localization of bovine thioredoxin determined by radioimmunoassay. *Biochemistry* 17: 4071–4077, 1978.
32. Kang SW, Baines IC, and Rhee SG. Characterization of a mammalian peroxiredoxin that contains one conserved cysteine. *J Biol Chem* 273: 6303–6311, 1998.
33. Kang SW, Chae HZ, Seo MS, Kim K, Baines IC, and Rhee SG. Mammalian peroxiredoxin isoforms can reduce hydrogen peroxide generated in response to growth factors and tumor necrosis factor-alpha. *J Biol Chem* 273: 6297–6302, 1998.
34. Kanzok SM, Schirmer RH, Turbachova I, Iozef R, and Becker K. The thioredoxin system of the malaria parasite *Plasmodium falciparum*. Glutathione reduction revisited. *J Biol Chem* 275: 40180–40186, 2000.
35. Kawazu S, Ikenoue N, Takemae H, Komaki-Yasuda K, and Kano S. Roles of 1-Cys peroxiredoxin in haem detoxification in the human malaria parasite *Plasmodium falciparum*. *FEBS J* 272: 1784–1791, 2005.
36. Kawazu S, Komaki K, Tsuji N, Kawai S, Ikenoue N, Hatabu T, Ishikawa H, Matsumoto Y, Himeno K, and Kano S. Molecular characterization of a 2-Cys peroxiredoxin from the human malaria parasite *Plasmodium falciparum*. *Mol Biochem Parasitol* 116: 73–79, 2001.
37. Kawazu S, Nozaki T, Tsuboi T, Nakano Y, Komaki-Yasuda K, Ikenoue N, Torii M, and Kano S. Expression profiles of peroxiredoxin proteins of the rodent malaria parasite *Plasmodium yoelii*. *Int J Parasitol* 33: 1455–1461, 2003.
38. Kawazu S, Tsuji N, Hatabu T, Kawai S, Matsumoto Y, and Kano S. Molecular cloning and characterization of a peroxiredoxin from the human malaria parasite *Plasmodium falciparum*. *Mol Biochem Parasitol* 109: 165–169, 2000.
39. Komaki-Yasuda K, Kawazu S, and Kano S. Disruption of the *Plasmodium falciparum* 2-Cys peroxiredoxin gene renders parasites hypersensitive to reactive oxygen and nitrogen species. *FEBS Lett* 547: 140–144, 2003.
40. Krnajski Z, Gilberger TW, Walter RD, Cowman AF, and Muller S. Thioredoxin reductase is essential for the survival of *Plasmodium falciparum* erythrocytic stages. *J Biol Chem* 277: 25970–25975, 2002.
41. Krnajski Z, Walter RD, and Müller S. Isolation and functional analysis of two thioredoxin peroxidases (peroxiredoxins) from *Plasmodium falciparum*. *Mol Biochem Parasitol* 113: 303–308, 2001.
42. Lee SP, Hwang YS, Kim YJ, Kwon KS, Kim HJ, Kim K, and Chae HZ. Cyclophilin a binds to peroxiredoxins and activates its peroxidase activity. *J Biol Chem* 276: 29826–29832, 2001.
43. Lee TH, Kim SU, Yu SL, Kim SH, Park do S, Moon HB, Dho SH, Kwon KS, Kwon HJ, Han YH, Jeong S, Kang

SW, Shin HS, Lee KK, Rhee SG, and Yu DY. Peroxiredoxin II is essential for sustaining life span of erythrocytes in mice. *Blood* 101: 5033–5038, 2003.
44. Lim YS, Cha MK, Yun CH, Kim HK, Kim K, and Kim IH. Purification and characterization of thiol-specific antioxidant protein from human red blood cell: A new type of antioxidant protein. *Biochem Biophys Res Commun* 199: 199–206, 1994.
45. Manevich Y, Feinstein SI, and Fisher AB. Activation of the antioxidant enzyme 1-Cys peroxiredoxin requires glutathionylation mediated by heterodimerization with pi GST. *Proc Natl Acad Sci U S A* 101: 3780–3785, 2004.
46. May JM, Mendiratta S, Hill KE, and Burk RF. Reduction of dehydroascorbate to ascorbate by the selenoenzyme thioredoxin reductase. *J Biol Chem* 272: 22607–22610, 1997.
47. Mieyal JJ, Starke DW, Gravina SA, Dothey C, and Chung JS. Thioltransferase in human red blood cells: purification and properties. *Biochemistry* 30: 6088–6097, 1991.
48. Mills GC. Hemoglobin catabolism, I: Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. *J Biol Chem* 229: 189–197, 1957.
49. Missirlis F, Ulschmid JK, Hirosawa-Takamori M, Gronke S, Schafer U, Becker K, Phillips JP, and Jackle H. Mitochondrial and cytoplasmic thioredoxin reductase variants encoded by a single *Drosophila* gene are both essential for viability. *J Biol Chem* 277: 11521–11526, 2002.
50. Montemartini M, Kalisz HM, Hecht HJ, Steinert P, and Flohé L. Activation of active-site cysteine residues in the peroxiredoxin-type tryparedoxin peroxidase of *Crithidia fasciculata*. *Eur J Biochem* 264: 516–524, 1999.
51. Mukherjee A and Martin SG. The thioredoxin system: A key target in tumour and endothelial cells. *Drug Discov Today* in press.
52. Muller S, Gilberger TW, Farber PM, Becker K, Schirmer RH, and Walter RD Recombinant putative glutathione reductase from *Plasmodium falciparum* exhibits thioredoxin reductase activity. *Mol Biochem Parasitol* 80: 215–219, 1996.
53. Muller S, Riedal HD, and Stremmel W. Direct evidence for catalase as the predominant hydrogen peroxide removing enzyme in human erythrocytes. *Blood* 90: 4973–4978, 1997.
54. Muller S. Redox and antioxidant systems of the malaria parasite *Plasmodium falciparum*. *Mol Microbiol* 53: 1291–1305, 2004.
55. Nagababu E, Chrest FJ, and Rifkind JM. Hydrogen-peroxide-induced heme degradation in red blood cells: the protective roles of catalase and glutathione peroxidase. *Biochim Biophys Acta* 1620: 211–217, 2003.
56. Neumann CA, Krause DS, Carman CV, Das S, Dubey DP, Abraham JL, Bronson RT, Fujiwara Y, Orkin SH, and Van Etten RA. Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. *Nature* 424: 561–565, 2003.
57. Nickel C. Strukturelle und funktionelle Charakterisierung von redoxaktiven Proteinen des Malariaparasiten *Plasmodium falciparum*. Dissertation, Universität Giessen, 2005.
58. Nickel C, Trujillo M, Rahlfs S, Deponte M, Radi R, and Becker K. *Plasmodium falciparum* 2-Cys peroxiredoxin reacts with plasmoredoxin and peroxynitrite. *Biol Chem* in press, 2005.
59. Padma P, Hozumi A, Ogawa K, and Inaba K. Molecular cloning and characterization of a thioredoxin/nucleoside diphosphate kinase related dynein intermediate chain from the ascidian, *Ciona intestinalis*. *Gene* 275: 177–183, 2001.
60. Papov VV, Gravina SA, Mieyal JJ, and Biemann K. The primary structure and properties of thioltransferase (glutaredoxin) from human red blood cells. *Protein Sci* 3: 428–434, 1994.
61. Patel-King RS, Benashki SE, Harrison A, and King SM. Two functional thioredoxins containing redox-sensitive vicinal dithiols from the *Chlamydomonas* outer dynein arm. *J Biol Chem* 271: 6283–6291, 1996.
62. Pauwels F, Vergauwen B, Vanrobaeys F, Devreese B, and Van Beeumen JJ. Purification and characterization of a chimeric enzyme from *Haemophilus influenzae* Rd that exhibits glutathione-dependent peroxidase activity. *J Biol Chem* 278: 16658–16666, 2003.
63. Rahlfs S and Becker K. Thioredoxin peroxidases of the malarial parasite *Plasmodium falciparum*. *Eur J Biochem* 268: 1404–1409, 2001.
64. Rahlfs S, Fischer M, and Becker K. *Plasmodium falciparum* possesses a classical glutaredoxin and a second, glutaredoxin-like protein with a PICOT homology domain. *J Biol Chem* 276: 37133–37140, 2001.
65. Rahlfs S, Nickel C, Deponte M, Schirmer RH, and Becker K. *Plasmodium falciparum* thioredoxins and glutaredoxins as central players in redox metabolism. *Redox Rep* 8: 246–250, 2003.
66. Rahlfs S, Schirmer RH, and Becker K. The thioredoxin system of *Plasmodium falciparum* and other parasites. *Cell Mol Life Sci* 59: 1024–1041, 2002.
67. Roos DS, Crawford MJ, Donald RG, Fraunholz M, Harb OS, He CY, Kissinger JC, Shaw MK, and Striepen B. Mining the *Plasmodium* genome database to define organellar function: what does the apicoplast do? *Phil Trans R Soc Lond B Biol Sci* 357: 35–46, 2002.
68. Rouhier N and Jacquot JP. Molecular and catalytic properties of a peroxiredoxin-glutaredoxin hybrid from *Neisseria meningitidis*. *FEBS Lett* 554: 149–153, 2003.
69. Rouhier N, Gelhaye E , and Jacquot JP. Glutaredoxin-dependent peroxiredoxin from poplar: protein-protein interaction and catalytic mechanism. *J Biol Chem* 277: 13609–13614, 2002.
70. Rouhier N, Gelhaye E, Sautiere PE, Brun A, Laurent P, Tagu D, Gerard J, de Fay E, Meyer Y, and Jacquot JP. Isolation and characterization of a new peroxiredoxin from poplar sieve tubes that uses either glutaredoxin or thioredoxin as a proton donor. *Plant Physiol* 127: 1299–1309, 2001.
71. Sakamoto A, Tsukamoto S, Yamamoto H, Ueda-Hashimoto M, Takahashi M, Suzuki H, and Morikawa H. Functional complementation in yeast reveals a protective role of chloroplast 2-Cys peroxiredoxin against reactive nitrogen species. *Plant J* 33: 841–851, 2003.
72. Sarma G, Nickel C, Rahlfs S, Fischer M, Becker K, and Karplus PA. Crystal structure of a novel *Plasmodium falci-*

parum 1-Cys peroxiredoxin. *J Mol Biol* 346: 1021–1034, 2005.
73. Seo MS, Kang SW, Kim K, Baines IC, Lee TH, and Rhee SG. Identification of a new type of mammalian peroxiredoxin that forms an intramolecular disulfide as a reaction intermediate. *J Biol Chem* 275: 20346–20354, 2000.
74. Siems WG, Sommerburg O, and Grune T. Erythrocyte free radical and energy metabolism. *Clin Nephrol* 53: 9–17, 2000.
75. Sienkiewicz N, Daher W, Dive D, Wrenger C, Viscogliosi E, Wintjens R, Jouin H, Capron M, Muller S, and Khalife J. Identification of a mitochondrial superoxide dismutase with an unusual targeting sequence in *Plasmodium falciparum. Mol Biochem Parasitol* 137: 121–132, 2004.
76. Sies H. Glutathione and its role in cellular functions. *Free Radic Biol Med* 27: 916–921, 1999.
77. Stuhlmeier KM, Kao JJ, Wallbrandt P, Lindberg M, Hammarstrom B, Broell H, and Paigen B. Antioxidant protein 2 prevents methemoglobin formation in erythrocyte hemolysates. *Eur J Biochem* 270: 334–341, 2003.
78. Sztajer H, Gamain B, Aumann KD, Slomianny C, Becker K, Brigelius-Flohé R, and Flohé L. The putative glutathione peroxidase gene of *Plasmodium falciparum* codes for a thioredoxin peroxidase. *J Biol Chem* 276: 7397–7403, 2001.
79. Torchinsky YM. *Sulphur in Proteins.* Oxford: Pergamon, 1981.
80. Trujillo M, Budde H, Pineyro MD, Stehr M, Robello C, Flohé L, and Radi R. *Trypanosoma brucei* and *Trypanosoma cruzi* tryparedoxin peroxidases catalytically detoxify peroxynitrite via oxidation of fast reacting thiols. *J Biol Chem* 279: 34175–34182, 2004.
81. Watson WH, Yang X, Choi YE, Jones DP, and Kehrer JP. Thioredoxin and its role in toxicology. *Toxicol Sci* 78: 3–14, 2004.
82. Weichsel A, Gasdaska JR, Powis G, and Montfort WR. Crystal structures of reduced, oxidized, and mutated human thioredoxins: Evidence for a regulatory homodimer. *Structure* 4: 735–751, 1996.
83. Williams CH Jr, Arscott LD, Müller S, Lennon BW, Ludwig ML, Wang P-F, Veine DM, Becker K, and Schirmer RH. Thioredoxin reductase: Two modes of catalysis have evolved. *Eur J Biochem* 267, 6110–6117, 2000.
84. Wood ZA, Poole LB, Hantgan RR, and Karplus PA. Dimers to doughnuts: redox-sensitive oligomerization of 2-cysteine peroxiredoxins. *Biochemistry* 41: 5493–5504, 2002.
85. Wood ZA, Schröder E, Robin Harris J, and Poole LB. Structure, mechanism and regulation of peroxiredoxins. *Trends Biochem Sci* 28: 32–40, 2003.
86. Yano K, Komaki-Yasuda K, Kobayashi T, Takemae H, Kita K, Kano S, and Kawazu S. Expression of mRNAs and proteins for peroxiredoxins in *Plasmodium falciparum* erythrocytic stage. *Parasitol Int* 54: 35–41, 2005.
87. Zhang P, Liu B, Kang SW, Seo MS, Rhee SG, and Obeid LM. Thioredoxin peroxidase is a novel inhibitor of apoptosis with a mechanism distinct from that of Bcl-2. *J Biol Chem* 272: 30615–30618, 1997.

Address reprint requests to:
Katja Becker
Interdisciplinary Research Center
Justus Liebig University
Heinrich-Buff-Ring 26–32
D-35392 Giessen, Germany

E-mail: becker.katja@gmx.de

Date of first submission to ARS Central, December 28, 2005; date of acceptance, January 22, 2006.

This article has been cited by:

1. Esther Jortzik, Katja Becker. 2012. Thioredoxin and glutathione systems in Plasmodium falciparum. *International Journal of Medical Microbiology* **302**:4-5, 187-194. [CrossRef]
2. Marcel Deponte. 2012. Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. *Biochimica et Biophysica Acta (BBA) - General Subjects* . [CrossRef]
3. Giovanna Boumis, Giorgio Giardina, Francesco Angelucci, Andrea Bellelli, Maurizio Brunori, Daniela Dimastrogiovanni, Fulvio Saccoccia, Adriana E. Miele. 2012. Crystal structure of Plasmodium falciparum thioredoxin reductase, a validated drug target. *Biochemical and Biophysical Research Communications* **425**:4, 806-811. [CrossRef]
4. R. Luise Krauth-Siegel , Alejandro E. Leroux . 2012. Low-Molecular-Mass Antioxidants in Parasites. *Antioxidants & Redox Signaling* **17**:4, 583-607. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
5. Michael C. Gretes , Leslie B. Poole , P. Andrew Karplus . 2012. Peroxiredoxins in Parasites. *Antioxidants & Redox Signaling* **17**:4, 608-633. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
6. Andrew J. Theobald, Iván Caballero, Isabel Coma, Gonzalo Colmenarejo, Concepcion Cid, Francisco-Javier Gamo, Martin J. Hibbs, Amy L. Bass, Daniel A. Thomas. 2012. Discovery and Biochemical Characterization of Plasmodium Thioredoxin Reductase Inhibitors from an Antimalarial Set. *Biochemistry* 120601130535005. [CrossRef]
7. Ashwani Sharma, Arvind Sharma, Sameer Dixit, Amit Sharma. 2011. Structural insights into thioredoxin-2: a component of malaria parasite protein secretion machinery. *Scientific Reports* **1**. . [CrossRef]
8. Hee-Young Yang, Joseph Kwon, Hoon-In Choi, Seong Hwa Park, Ung Yang, Hyang-Rim Park, Lina Ren, Kyoung-Jin Chung, Youn U. Kim, Byung-Ju Park, Sang-Hun Jeong, Tae-Hoon Lee. 2011. In-depth analysis of cysteine oxidation by the RBC proteome: Advantage of peroxiredoxin II knockout mice. *PROTEOMICS* n/a-n/a. [CrossRef]
9. Shin-ichiro Kawazu, Hitoshi Takemae, Kanako Komaki-Yasuda, Shigeyuki Kano. 2010. Target proteins of the cytosolic thioredoxin in Plasmodium falciparum. *Parasitology International* **59**:2, 298-302. [CrossRef]
10. Fátima Nogueira, Amalia Diez, Azar Radfar, Susana Pérez-Benavente, Virgilio E. do Rosario, Antonio Puyet, José M. Bautista. 2010. Early transcriptional response to chloroquine of the Plasmodium falciparum antioxidant defence in sensitive and resistant clones#. *Acta Tropica* **114**:2, 109-115. [CrossRef]
11. Miriam Urscher, Jude M. Przyborski, Masaya Imoto, Marcel Deponte. 2010. Distinct subcellular localization in the cytosol and apicoplast, unexpected dimerization and inhibition of Plasmodium falciparum glyoxalases. *Molecular Microbiology* **76**:1, 92-103. [CrossRef]
12. Claudia Elisabeth Munte, Katja Becker, Rolf Heiner Schirmer, Hans Robert Kalbitzer. 2009. NMR assignments of oxidised thioredoxin from Plasmodium falciparum. *Biomolecular NMR Assignments* **3**:2, 159-161. [CrossRef]
13. Miho Tanaka, Tatsuya Sakurai, Naoaki Yokoyama, Noboru Inoue, Shin-ichiro Kawazu. 2009. Cloning and characterization of peroxiredoxin in Babesia bovis. *Parasitology Research* **105**:5, 1473-1477. [CrossRef]
14. S. Koncarevic, P. Rohrbach, M. Deponte, G. Krohne, J. H. Prieto, J. Yates, S. Rahlfs, K. Becker. 2009. The malarial parasite Plasmodium falciparum imports the human protein peroxiredoxin 2 for peroxide detoxification. *Proceedings of the National Academy of Sciences* **106**:32, 13323-13328. [CrossRef]
15. John R. Gallagher, Sean T. Prigge. 2009. Plasmodium falciparum acyl carrier protein crystal structures in disulfide-linked and reduced states and their prevalence during blood stage growth. *Proteins: Structure, Function, and Bioinformatics* NA-NA. [CrossRef]
16. Jeralyn D. Haraldsen, Gu Liu, Catherine H. Botting, Jeffrey G. A. Walton, Janet Storm, Timothy J. Phalen, Lai Yu Kwok, Dominique Soldati-Favre, Nicholas H. Heintz, Sylke Müller, Nicholas J. Westwood, Gary E. Ward. 2009. Identification of conoidin A as a covalent inhibitor of peroxiredoxin II. *Organic & Biomolecular Chemistry* **7**:15, 3040. [CrossRef]
17. D. Parsonage, P. A. Karplus, L. B. Poole. 2008. Reactive Oxygen Species Special Feature: Substrate specificity and redox potential of AhpC, a bacterial peroxiredoxin. *Proceedings of the National Academy of Sciences* **105**:24, 8209-8214. [CrossRef]
18. Azar Radfar, Amalia Diez, José M. Bautista. 2008. Chloroquine mediates specific proteome oxidative damage across the erythrocytic cycle of resistant Plasmodium falciparum. *Free Radical Biology and Medicine* **44**:12, 2034-2042. [CrossRef]
19. Kazuhiko Yano, Hitoshi Otsuki, Meiji Arai, Kanako Komaki-Yasuda, Takafumi Tsuboi, Motomi Torii, Shigeyuki Kano, Shin-Ichiro Kawazu. 2008. Disruption of the Plasmodium berghei 2-Cys peroxiredoxin TPx-1 gene hinders the sporozoite development in the vector mosquito. *Molecular and Biochemical Parasitology* **159**:2, 142-145. [CrossRef]

20. A SANNELLA, A CASINI, C GABBIANI, L MESSORI, A BILIA, F VINCIERI, G MAJORI, C SEVERINI. 2008. New uses for old drugs. Auranofin, a clinically established antiarthritic metallodrug, exhibits potent antimalarial effects in vitro: Mechanistic and pharmacological implications. *FEBS Letters* **582**:6, 844-847. [CrossRef]
21. S KAWAZU, K KOMAKIYASUDA, H OKU, S KANO. 2008. Peroxiredoxins in malaria parasites: Parasitologic aspects. *Parasitology International* **57**:1, 1-7. [CrossRef]
22. Christophe Morin, Tatiana Besset, Jean-Claude Moutet, Martine Fayolle, Margit Brückner, Danièle Limosin, Katja Becker, Elisabeth Davioud-Charvet. 2008. The aza-analogues of 1,4-naphthoquinones are potent substrates and inhibitors of plasmodial thioredoxin and glutathione reductases and of human erythrocyte glutathione reductase. *Organic & Biomolecular Chemistry* **6**:15, 2731. [CrossRef]
23. Irwin W. ShermanReferences **67**, 325-394. [CrossRef]
24. Ivan Usynin, Christian Klotz, Ute Frevert. 2007. Malaria circumsporozoite protein inhibits the respiratory burst in Kupffer cells. *Cellular Microbiology* **9**:11, 2610-2628. [CrossRef]
25. Jan M. Woynarowski, Miriam Krugliak, Hagai Ginsburg. 2007. Pharmacogenomic analyses of targeting the AT-rich malaria parasite genome with AT-specific alkylating drugs. *Molecular and Biochemical Parasitology* **154**:1, 70-81. [CrossRef]
26. Mario Milani, Emanuela Balconi, Alessandro Aliverti, Eloise Mastrangelo, Frank Seeber, Martino Bolognesi, Giuliana Zanetti. 2007. Ferredoxin-NADP+ Reductase from Plasmodium falciparum Undergoes NADP+-dependent Dimerization and Inactivation: Functional and Crystallographic Analysis. *Journal of Molecular Biology* **367**:2, 501-513. [CrossRef]