Sequence Analysis of the Tryparedoxin Peroxidase Gene from
Crithidia fasciculata and Its Functional Expression in
Escherichia coli*

(Received for publication, September 9, 1997, and in revised form, December 15, 1997)

Marisa Montemartini‡‡, Everson Nogoceke‡‡, Mahavir Singh, Peter Steinert, Leopold Flohé, and Henryk M. Kalisz‡‡**

From the ‡‡Gesellschaft für Biotechnologische Forschung (GBF) mbH, Mascheroder Weg 1, D-38124 Braunschweig, Germany and the ‡‡Department of Physiological Chemistry, Technical University of Braunschweig, c/o GBF, Mascheroder Weg 1, D-38124 Braunschweig, Germany

Tryparedoxin peroxidase from Crithidia fasciculata is an essential component of the trypantothione-dependent hydroperoxide metabolism in the trypanosomatids (Nogoceke, E., Gommel, D. U., Kieß, M., Kalisz, H. M., and Flohé, L. (1997) Biol. Chem. 378, 827–836). The tryparedoxin peroxidase gene and its flanking regions have been isolated and sequenced from a C. fasciculata genomic DNA library. It consists of an open reading frame of 864 base pairs encoding a protein of 188 amino acid residues. The gene, modified to encode 6 additional histidine residues, was expressed in Escherichia coli and the recombinant protein was purified to homogeneity by metal chelating chromatography. Recombinant tryparedoxin peroxidase has a subunit molecular mass of 21884 ± 22 and contains two isoforms of pI 6.2 and 6.3. It exhibits a kinetic pattern identical to that of the authentic tryparedoxin peroxidase and has a similar specific activity of 2.51 units mg⁻¹. The enzyme unequivocally belongs to the peroxiredoxin family of proteins, whose members have been found in all phyla. A phylogenetic tree comprising 47 protein and DNA sequences showed tryparedoxin peroxidase and a homologous Trypanosoma brucei sequence to form a distinct molecular clade. The consensus sequence: x₄Ax₅₋₆Fx₆Gx₇Vx₈-Fx₉Fx₁₀Fx₁₁FVPCPTEx₁₂Sx₁₃Dx₁₄₋₁₅Dx₁₅₋₁₆Gx₁₇Rxₓ₁₈Fx₉-Dx₁₉Ax₁ₓ₂-Gx₃₋₄Cx₅₋₆Vx₇, was demonstrated by alignment of the sequences of tryparedoxin peroxidase and 8 other peroxiredoxins with established peroxidase function.

Tryparedoxin peroxidase has recently been identified as a constituent of the complex peroxidase system in the trypanosomatid Crithidia fasciculata (1). In these parasitic protozoa hydroperoxides are reduced at the expense of NADPH by means of a cascade of three oxidoreductases: the flavoprotein trypanothione reductase, the thioredoxin-related tryparedoxin, and tryparedoxin peroxidase (Fig. 1). The first enzyme of the cascade is homologous to glutathione reductase and thioredoxin reductase (2), which are involved in NADPH-dependent hydroperoxide reduction in other species (3). The other components of the trypanosomatid system also belong to protein families occasionally constituting peroxidase systems. Preliminary amino acid sequencing data indicated that tryparedoxin peroxidase is phylogenetically related to thioredoxin (1), whereas the tryparedoxin peroxidase belongs to the peroxiredoxins (1) comprising the thioredoxin peroxidases of yeast and mammals (4) and the alkyl hydroperoxide reductases of bacteria (5).

The unique feature of the trypanosomatid peroxidase system is its dependence on the peculiar redox mediator trypanothione which so far has not been discovered in any species outside the Trypanosomatidae. Its biosynthesis from spermidine and glutathione requires two distinct enzymes, glutathionylspermidine synthetase (6) and trypanothione synthetase (7). With a cascade of oxidoreductases plus the redox mediator trypanothione and the two auxiliary enzymes for its synthesis, the trypanosomatids have developed the most complicated system for the removal of hydroperoxides so far discovered in nature. This is not to imply a particular efficiency or robustness of the system. On the contrary, trypanosomatids are reported to be highly susceptible to oxidative stress (8). Correspondingly, their extraordinary metabolism is being discussed as a potential target area of specific trypanocidal agents (1, 9, 10).

The present possibilities available for the treatment of trypanosomal diseases, such as Chagas disease, African sleeping sickness, and the various forms of leishmaniasis, necessitate improvement. We (1, 6), like many others (11–14), have therefore embarked on the identification and characterization of potential molecular targets typical of the trypanosomatids. Here we report for the first time the full-length DNA, deduced amino acid sequence, and expression of a tryparedoxin peroxidase and its relatedness to peroxiredoxins with established or unknown functions.

EXPERIMENTAL PROCEDURES

Cell Culture and DNA Extraction—C. fasciculata (HS6) was grown as described by Shim and Fairlamb (15). The cells were harvested by centrifugation for 15 min at 7000 rpm, washed twice with saline solution (0.9% NaCl), and resuspended in 5 ml of buffer (50 mM Tris-HCl, 100 mM EDTA, 15 mM NaCl, 0.5% SDS, 100 μg ml⁻¹ proteinase K, pH 8.0). Resuspended cells were preincubated at 50°C for 40 min. The genomic DNA was extracted twice with equivalent volumes of phenol (incubation: 60°C for 45 min; centrifugation: 20 min, 4500 rpm) followed by phenol:chloroform:isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol extraction (24:1). Genomic DNA was precipitated with sodium acetate and ethanol.

Primers, Hybridization Probes, and Sequence Analysis—Based upon peptide sequences of tryparedoxin peroxidase (1), degenerate oligodeoxyribonucleotides 5’-TCAGATCCGAYATGGCSCTATGC-3’ and 5’-
The tryparedoxin peroxidase gene contained in the cloned 1.5-kb library gave positive PCR signals for tryparedoxin peroxidase and were sequenced peptide fragments obtained by the program DARWIN (16). The EMBL and SWISSPROT data bases were screened for tryparedoxin peroxidase homologous nucleotide or peptide sequences using BLAST and FASTA. Multiple alignments were performed by the program PILEUP (GGG Wisconsin Program Package, Version 9.0-OpenVMS) using default parameters. The polypeptide sequences of data base entries comprising complete coding regions were compared by the program DARWIN (16).

RESULTS

Isolation and Sequencing of the Tryparedoxin Peroxidase Gene from C. fasciculata—Sequenced peptide fragments obtained from purified tryparedoxin peroxidase of C. fasciculata could be aligned along the established deduced amino acid sequence of the thiol-specific antioxidant protein of yeast (1). This enabled appropriate degenerate PCR primers to be designed for the generation of a PCR product from the C. fasciculata genomic DNA. This PCR product, which was 0.4 kb long (see Fig. 3), was subsequently used to screen a genomic library for inserts containing the full-length DNA encoding the tryparedoxin peroxidase. A clone containing a 15-kb insert with the sequence of the thiol-specific antioxidant protein of yeast (1).
the presumed tryparedoxin peroxidase gene was isolated and sequenced. This, however, led to the detection of equal quantities of different nucleotides at several positions toward the 3’ end of the gene, implying the presence of similar but not identical genes in the insert. This observation was not unexpected since the genome of the Trypanosomatidae is known to contain repetitive structural genes separated by intergenic sequences (17–19). The λ-clone was consequently digested with the restriction enzyme SacI and a Southern blot was performed. Three fragments belonging to the insert (1.1, 1.5, and 11 kb) gave positive hybridization signals with the labeled PCR product. Each of the three fragments was subcloned into pBlueScript II KS(+) phagemids and sequenced. The general sequencing strategy is shown in Fig. 2 using the 1.5-kb fragment as an example.

The 11-kb fragment contained the information coding for the previously sequenced peptides of tryparedoxin peroxidase (Fig. 3). Nevertheless, as with the 15-kb insert, the bases at positions 542, 548, 551, 556, 557, 560, 563, 564, and 565 remained ambiguous suggesting the presence of more than one gene in this fragment. The nucleotide sequence shown in Fig. 3 was confirmed by resequencing the 1.5-kb fragment which contained an open reading frame largely identical to the one of the

---

**Fig. 2. Restriction map and sequencing strategy for the tryparedoxin peroxidase gene.** The region shown is that of the 1.5-kb fragment. The open reading frame for the tryparedoxin peroxidase gene (TXNPs) is indicated as an open box. The arrows show the directions and approximate positions of the primers used for sequencing. The two terminal arrows indicate primers hybridized to the cloning vector.

---

**Fig. 3.** Nucleotide and deduced amino acid sequences of the tryparedoxin peroxidase gene from *C. fasciculata*. The arrows delimit the PCR product used to screen the genomic library. The start and stop codons are in **bold**, as is the asparagine residue which was replaced by a threonine residue in direct peptide sequencing. Sequences confirmed by protein sequence analysis are **underlined**. The position of the SacI site in the 1.1-kb fragment is **heavily underlined**. The differences in the coding region between the 1.5- and 11-kb fragments, and in the 5’-flanking region between the 1.5- and 11-kb fragments are shown in **brackets**. The AG consensus splice leader sites and the poly pyrimidine-rich tract are **double underlined**.
11-kb fragment except for the presence of an additional cytosine at position 30. As a consequence of the resulting frameshift, the deduced amino acid sequence no longer complied with the established peptide sequences. Hence, the 1.5-kb fragment contained a pseudogene. The 1.1-kb fragment also contained an open reading frame but encoded only part of the tryparedoxin peroxidase since a SacI restriction site was present at position 472–477. This reading frame was therefore not sequenced to completion. All the amino acid sequences determined for the peptide fragments of the C. fasciculata tryparedoxin peroxidase matched those predicted from the DNA shown in Fig. 3, except for positions 406–408 where an asparagine is encoded but a threonine was determined by amino acid sequencing. This asparagine was found in all of the sequenced DNA fragments and in the PCR product.

When comparing the 5′-flanking region of the 1.5-kb fragment with one of the 1.1-kb fragments, only minor differences were found (Fig. 3). In both fragments two possible consensus dinucleotides AG, which represent potential splice leader acceptor sites, are found in positions −52 and −125. However, only the one in position −125 is preceded by a pyrimidine-rich tract which is necessary for trans-splicing (20).

Expression of the Tryparedoxin Peroxidase Gene in E. coli BL21(DE3)—The identification of multiple open reading frames encoding tryparedoxin peroxidase required confirmation of functionality of the sequence shown in Fig. 3. For this purpose the 1.5-kb fragment exhibiting least sequence ambiguities appeared most appropriate except for the inserted frameshift mutation. Therefore, the frameshifting extra cytosine base at position 30 was deleted, the region coding for the C-terminal amino acids was modified as described under “Experimental Procedures” to facilitate purification of the recombinant tryparedoxin peroxidase on a Ni²⁺-binding resin. Tryparedoxin peroxidase bound to the resin, whereas most of the impurities either did not bind or were washed from the column with the binding buffer containing 100 mM imidazole. Tryparedoxin peroxidase eluted at 500 mM imidazole and was shown to be homogeneous by SDS-PAGE and subsequent silver staining (Fig. 6A). N-terminal sequencing of this protein showed the initial methionine to be missing and allowed us to confirm the first 30 amino acids. The expressed tryparedoxin peroxidase had nearly the same molecular mass of about 21,000 as the authentic tryparedoxin peroxidase in SDS-PAGE (Fig. 6A). Matrix-assisted laser desorption/ionization-mass spectrometry analysis demonstrated the molecular mass of the recombinant enzyme to be 21,884 ± 22. The difference in the molecular mass of 1,004 to the authentic tryparedoxin peroxidase (1) corresponded to the additional amino acids (leucine, glutamate, and 6 histidines) added at the C-terminal end of the expressed protein enabled simple purification of the recombinant tryparedoxin peroxidase on a Ni²⁺-binding resin. Tryparedoxin peroxidase bound to the resin, whereas most of the impurities either did not bind or were washed from the column with the binding buffer containing 100 mM imidazole. Tryparedoxin peroxidase eluted at 500 mM imidazole and was shown to be homogeneous by SDS-PAGE and subsequent silver staining (Fig. 6A). N-terminal sequencing of this protein showed the initial methionine to be missing and allowed us to confirm the first 30 amino acids. The expressed tryparedoxin peroxidase had nearly the same molecular mass of about 21,000 as the authentic tryparedoxin peroxidase in SDS-PAGE (Fig. 6A). Matrix-assisted laser desorption/ionization-mass spectrometry analysis demonstrated the molecular mass of the recombinant enzyme to be 21,884 ± 22. The difference in the molecular mass of 1,004 to the authentic tryparedoxin peroxidase (1) corresponded to the additional amino acids (leucine, glutamate, and 6 histidines) added at the C-terminal end of the recombinant enzyme. Whereas the authentic tryparedoxin peroxidase contained several isoforms ranging from pI 4.9 to 5.8, the recombinant protein showed two bands of pI 6.2 and 6.3. The higher alkalinity results from the additional histidines residues.

The pure recombinant enzyme had a specific activity of 2.51 units/mg compared with 5.83 units/mg for the authentic enzyme. This difference may be due to the additional residues at the C-terminal end of the recombinant tryparedoxin peroxidase. The kinetic analysis of the recombinant protein revealed a kinetic pattern identical to that of the authentic tryparedoxin peroxidase, i.e. a ping-pong mechanism with infinite maximum velocities and Michaelis constants. Initial velocities when fitted to the general Dalziel equation for bisubstrate reactions, where ROOH = hydroperoxide and TXN = tryparedoxin,

\[
\frac{[E_0]}{v} = \phi_0 + \frac{\phi_1}{[ROOH]} + \frac{\phi_2}{[TXN]} + \frac{\phi_{1,2}}{[ROOH][TXN]} \quad (\text{Eq. 1})
\]

revealed that the coefficients \(\phi_0\) and \(\phi_{1,2}\) were zero. The kinetic parameters obtained with the pure recombinant tryparedoxin

---

**Fig. 4. Structure of the plasmid vector used for the expression of tryparedoxin peroxidase in E. coli.** The restriction sites (XhoI and NdeI) where the tryparedoxin peroxidase gene (TXNPx) was inserted are shown. His, histidine tag coding sequence; Kan r, kanamycin resistance coding sequence; lacI, LacI coding sequence; ori, pBR322 origin; T7 prom, T7 promoter.

**Fig. 5. Tryparedoxin peroxidase activity in the supernatants of E. coli pET24 and E. coli pET/TXNPx.** LB medium containing 30 μg of kanamycin/ml was inoculated with a single colony and the E. coli cells were grown at 36 °C, 180 rpm. When OD₆₀₀ was 0.3 (time 0), 10-ml aliquots were withdrawn at hourly intervals, resuspended in 2 ml of 50 mM Tris-HCl, pH 8.0, 1 mM EDTA buffer, sonicated, and centrifuged. Tryparedoxin peroxidase activity was measured in the supernatant as stated under “Experimental Procedures.” The arrow indicates the time of induction by isopropyl-β-D-thiogalactopyranoside addition. ●, E. coli pET24; ○, E. coli pET/TXNPx.
pET/TpodH6 cells 5 h after induction; lane 3, tryparedoxin peroxidase. C. fasciculata polyvinylidene difluoride membranes. Whole rabbit serum (1:250 dilution) containing antibodies raised against pure from C. fasciculata BL21(DE3) pET/TpodH6 cells 5 h after induction; lane 2, supernatant of E. coli BL21(DE3) pET/TpodH6 cells before induction; lane 3, purified recombinant tryparedoxin peroxidase from C. fasciculata; lane 5, molecular weight standards. B, Western blotting was performed by electrotransferring proteins from SDS gels onto polyvinylidene difluoride membranes. Whole rabbit serum (1:250 dilution) containing antibodies raised against pure C. fasciculata tryparedoxin peroxidase was used as primary antibody and anti-rabbit goat antibodies (Sigma) as secondary antibody for the immunodetection of recombinant tryparedoxin peroxidase. Lane 1, supernatant of E. coli BL21(DE3) pET/TpodH6 cells before induction; lane 2, supernatant of E. coli BL21(DE3) pET/TpodH6 cells 5 h after induction; lane 3, purified recombinant tryparedoxin peroxidase.

FIG. 6. Analysis of tryparedoxin peroxidase expressed in E. coli by SDS-PAGE and Western blotting. A, SDS-PAGE was done under reducing conditions in 8–25% gradient gels on a Pharmacia Phast System and the proteins were visualized by silver staining according to the manufacturers’ recommendations. Lane 1, supernatant of E. coli BL21(DE3) pET/TpodH6 cells before induction; lane 2, supernatant of E. coli BL21(DE3) pET/TpodH6 cells 5 h after induction; lane 3, purified recombinant tryparedoxin peroxidase; lane 4, authentic tryparedoxin peroxidase from C. fasciculata; lane 5, molecular weight standards. B, Western blotting was performed by electrotransferring proteins from SDS gels onto polyvinylidene difluoride membranes. Whole rabbit serum (1:250 dilution) containing antibodies raised against pure C. fasciculata tryparedoxin peroxidase was used as primary antibody and anti-rabbit goat antibodies (Sigma) as secondary antibody for the immunodetection of recombinant tryparedoxin peroxidase.

TABLE I

| Kinetic parameter | Recombinant enzyme | Authentic enzyme |
|-------------------|--------------------|------------------|
| $\phi_1$ (10^{-6} M s) | 11.9 ± 1.2 | 5.9 ± 1.8 |
| $k_1$ (10^{5} M^{-1} s^{-1}) | 0.8 ± 0.1 | 1.8 ± 0.5 |
| $\phi_2$ (10^{-7} M s) | 1.1 ± 0.1 | 5.1 ± 0.7 |
| $k_2$ (10^{6} M^{-1} s^{-1}) | 9.4 ± 0.8 | 2.0 ± 0.3 |

Data are the means of six independent measurements. All values are calculated per subunit concentration. The parameters for the authentic enzyme are taken from Nogoeke et al. (1).

peroxidase and the authentic tryparedoxin peroxidase isolated from C. fasciculata are compared in Table I.

Comparison with Homologous Sequences—Based on sequence analysis, tryparedoxin peroxidase belongs to the family of peroxiredoxins (1). More than 50 homologous sequences were detected in the SwissProt and EMBL data banks, which comprised various bacterial alkyl hydroperoxide reductases (5), the thioredoxin peroxidases from yeast (21) and mammals (4) previously called TSA proteins (22–24), NKEF-thioredoxin peroxidases from yeast (21) and mammals (4) previously called TSA proteins (22–24), NKEF-thioredoxin peroxidases from yeast (21) and mammals (4), and yeast (21) and mammals (4), the tryparedoxin peroxidase and the authentic tryparedoxin peroxidase isolated from C. fasciculata are compared in Table I.

Out of these protein and DNA sequences 47 were selected to construct an unreooted phylogenetic tree, whereby the selection was based on (i) completeness of the published sequence, (ii) similarity in size, and (iii) likelihood of homology with a maximum FASTA E-score of 4.5. In the thereby obtained phylogenetic tree (Fig. 7), tryparedoxin peroxidase Q together with the homologous T. brucei sequence R presents a distinct molecular clade branching off between the sequences of the metazoan (A–P), including one of the known helminth sequences (T) on the one side with the rest, comprising the sequences of entamoeba (S), yeast (α, β), plants (γ–μ), bacteria (α–o), and, surprisingly, another helminth (U), on the other side.

Sequence alignments were further used to generate ideas about the potential functional relevance of particular amino acid residues. For this purpose we aligned a total of nine sequences for which a peroxidase function had been either clearly established or suggested from biological experiments (Fig. 8). These were the thioredoxin peroxidases of Saccharomyces cerevisiae (21) and Homo sapiens (4), the tryparedoxin peroxidase described here, and the alkyl hydroperoxide reductases from Salmonella typhimurium (5), E. coli (27), Corynebacterium diphtheriae (28), Bacillus subtilis (29), and Mycobacterium tuberculosis (30). Although the similarities between these sequences of poorly related species ranged from 29 to 77%, a total of 21 residues were strictly conserved in homologous positions. The overall consensus sequence is: $x_1A_{5–6}^\alpha – F_{X_2}G_{X_3}V_{X_4}F_{X_5}P_{X_6}F_{VPCPT}E_{X_9}S_{X_{10}}D_{X_{11}}W_{X_{12–13}}D_{X_{15–16}}G_{X_{20}}R_{X_2}F_{X_3}D_{X_7}A_{X_{11–12}}C_{X_{13–14}}W_{X_{15}}$.

The first cysteine residue is integrated into a completely conserved VCP motif which is widespread in the whole peroxiredoxin family. The C-proximal cysteine also forms a VCP motif in seven of the aligned peroxidases, whereas in M. tuberculosis the motif is altered to LCA. The C-proximal VCP and LCA motifs, respectively, are separated by two residues from the last conserved tryptophan. In the thioredoxin peroxidase of yeast the corresponding region reads VLFCNW.

DISCUSSION

We have cloned a genomic DNA fragment from C. fasciculata that encodes multiple copies of the entire sequence of tryparedoxin peroxidase, which is an essential constituent of the trypanosomatid peroxidase metabolism (1). The reading frame is very similar to a DNA sequence of T. brucei rhodesiense encoding a protein of unknown function (26). The high degree of similarity suggests that this trypanosomal protein is also a tryparedoxin peroxidase. However, the multiplication of genes in the trypanosomatids, which may lead to pseudogenes, precludes a definite functional interpretation of DNA sequences without experimental evidence. In the case of the C. fasciculata DNA sequence shown in Fig. 3, its functional relevance was unambiguously established by heterologous expression and comparison of the gene product with the authentic tryparedoxin peroxidase.
Tryparedoxin peroxidase unequivocally belongs to the family of peroxiredoxins. This protein family is obviously widespread in nature. The phylogenetic tree showing the molecular evolution of peroxiredoxins practically covers all phyla from bacteria to vertebrates but it does not simply reflect the phylogenetic divergence of the species. The sequence of the parasitic plathelmint Brugia malayi (T), for example, debranches from protozoal and vertebrate sub-trees, whereas another plathelmint protein (U) belongs to a plant sub-tree. There are two distinct molecular clades found in chlorophyta plus a separate type in the diatomean alga Odonthella, each diverging at distant points from bacterial branches. Similarly, one of the two yeast peroxiredoxins debranches from a plant branch, which in turn diverges from bacterial ancestors. This puzzling situation suggests multiple gene acquisition by means of endosymbiosis. Such gene transfer has also been implicated for various Euglenozoa, including the Trypanosomatidae (31). Even the cytosolic GapC of T. brucei and Leishmania mexicana, for example, is believed to be acquired from endosymbiotic γ-purple bacteria and to be secondarily integrated into the
The nuclear genome of the trypanosomatids. The debranching point of the two trypanosomatid peroxiredoxins, however, does not lend any support to speculations about secondary gene acquisition. Debranching between the metazoa and yeast, bacteria, and plants, the molecular evolution of the trypanosomal peroxiredoxins appears congruent with taxonomical development. Molecular evolution has given ample room for functional diversification within the peroxiredoxin family and it cannot be uncritically presumed that all peroxiredoxins are peroxidases or “antioxidant proteins.” Conceivably, many of the

**Fig. 8.** GCG alignment of peroxiredoxins with known peroxidase activity. Gap creation penalty = 12; gap extension penalty = 4. Strictly conserved amino acids and the highly conserved ones forming the degenerate second VCP motif are marked in **bold**. **AHPC**, alkyl hydroperoxidase C, **TXNPx**, tryparedoxin peroxidase; **Bsub**, *B. subtilis*; **Cfas**, *C. fasciculata*; **Cdip**, *C. diphtheriae*; **Ecol**, *E. coli*; **Hsap**, *H. sapiens*; **Mtub**, *M. tuberculosis*; **Scer**, *S. cerevisiae*; **Styp**, *S. typhimurium*.

| TXNPX_{Cfas} | 1 | ~~~~~~~~~~M SSGAAKLNHP APEFDDWALM PNGTFFKVSL SSYKGYVVL |
| NKEF_{Hsap} | 1 | ~~~~~~~~~~M SSGAAKLNHP APEFDDWALM PNGTFFKVSL SSYKGYVVL |
| NKEF_{Hsap} | 1 | ~~~~~~~~~~M SSGAAKLNHP APEFDDWALM PNGTFFKVSL SSYKGYVVL |
| TXA_{Gser} | 1 | ~~~~~~~~~~M MAAQVQOKQ APTFFKTVVM DGYFEDVSL DSKGYKLYVL |
| AHPC_{Gsub} | 1 | ~~~~~~~~~~M MAAQVQOKQ APTFFKTVVM DGYFEDVSL DSKGYKLYVL |
| AHPC_{Gsub} | 1 | ~~~~~~~~~~M MAAQVQOKQ APTFFKTVVM DGYFEDVSL DSKGYKLYVL |
| AHPC_{Gsub} | 1 | ~~~~~~~~~~M MAAQVQOKQ APTFFKTVVM DGYFEDVSL DSKGYKLYVL |
| AHPC_{Gsub} | 1 | ~~~~~~~~~~M MAAQVQOKQ APTFFKTVVM DGYFEDVSL DSKGYKLYVL |
| TXNPX_{Cfas} | 42 | FFYPMDFTFV CPEIIQFSD DAKRFAEINT EVISCSDCDE YSHLQWTSDV |
| NKEF_{Hsap} | 42 | FFYPMDFTFV CPEIIQFSD DAKRFAEINT EVISCSDCDE YSHLQWTSDV |
| NKEF_{Hsap} | 41 | FFYPMDFTFV CPEIIQFSD DAKRFAEINT EVISCSDCDE YSHLQWTSDV |
| TXA_{Gser} | 38 | AFPIPAFTVF CPEIIAFAE AAKFEQOGA QVLFASTDSE YSSLAWHNI |
| AHPC_{Gsub} | 37 | CYPAFDSFVF CPELEDLQF QYALKELGVQ EVYSVSDTDE FTHKAWHSSS |
| AHPC_{Gsub} | 37 | CYPAFDSFVF CPELEDLQF QYALKELGVQ EVYSVSDTDE FTHKAWHSSS |
| AHPC_{Gsub} | 37 | CYPAFDSFVF CPELEDLQF QYALKELGVQ EVYSVSDTDE FTHKAWHSSS |
| AHPC_{Gsub} | 37 | CYPAFDSFVF CPELEDLQF QYALKELGVQ EVYSVSDTDE FTHKAWHSSS |
| TXNPX_{Cfas} | 92 | RKKGGLGMPA IPLNADTKGG IRAAYGVLDE DSGVYARGVF IIDPGKILQR |
| NKEF_{Hsap} | 92 | RKKGGLGMPA IPLNADTKGG IRAAYGVLDE DSGVYARGVF IIDPGKILQR |
| NKEF_{Hsap} | 91 | RKKGGLGMPA IPLNADTKGG IRAAYGVLDE DSGVYARGVF IIDPGKILQR |
| TXA_{Gser} | 88 | RKKGGLGMPA IPLNADTKGG IRAAYGVLDE DSGVYARGVF IIDPGKILQR |
| AHPC_{Gsub} | 87 | EKEIISKIT YAMIGDSQT ISRFDVLDE ETGLASTGTF IIDPGKIR |
| AHPC_{Gsub} | 87 | EKEIISKIT YAMIGDSQT ISRFDVLDE ETGLASTGTF IIDPGKIR |
| AHPC_{Gsub} | 87 | EKEIISKIT YAMIGDSQT ISRFDVLDE ETGLASTGTF IIDPGKIR |
| AHPC_{Gsub} | 99 | .QHNDKTLTP FPLMLSAKRE LSQAQGVLNA DGVAFNVT DGVFNQIE |
| AHPC_{Gsub} | 99 | .QHNDKTLTP FPLMLSAKRE LSQAQGVLNA DGVAFNVT DGVFNQIE |
| TXNPX_{Cfas} | 142 | IIINDMDPIGR NVEEVIRLVE ALQFVEEHI. EV..CPANWK KDAAKKEG |
| NKEF_{Hsap} | 142 | IIINDMDPIGR NVEEVIRLVE ALQFVEEHI. EV..CPANWK KDAAKKEG |
| NKEF_{Hsap} | 141 | IIINDMDPIGR NVEEVIRLVE ALQFVEEHI. EV..CPANWK KDAAKKEG |
| TXA_{Gser} | 138 | IIINDMDPIGR NVEEVIRLVE ALQFVEEHI. EV..CPANWK KDAAKKEG |
| AHPC_{Gsub} | 134 | VEINAGGIGR DASLNVKVK AAQYVRQPNG EV..CPANWK EGGSTTLTSL |
| AHPC_{Gsub} | 134 | VEINAGGIGR DASLNVKVK AAQYVRQPNG EV..CPANWK EGGSTTLTSL |
| AHPC_{Gsub} | 134 | VEINAGGIGR DASLNVKVK AAQYVRQPNG EV..CPANWK EGGSTTLTSL |
| AHPC_{Gsub} | 147 | VASATGVSVR NVDEVLRLVD ALQ...SD EL..CANCNR KGDTPTLDA |
| AHPC_{Gsub} | 147 | VASATGVSVR NVDEVLRLVD ALQ...SD EL..CANCNR KGDTPTLDA |
| TXNPX_{Cfas} | 189 | ~~~~~~~~~~M SSGAAKLNHP APEFDDWALM PNGTFFKVSL SSYKGYVVL |
| NKEF_{Hsap} | 189 | ~~~~~~~~~~M SSGAAKLNHP APEFDDWALM PNGTFFKVSL SSYKGYVVL |
| NKEF_{Hsap} | 188 | ~~~~~~~~~~M SSGAAKLNHP APEFDDWALM PNGTFFKVSL SSYKGYVVL |
| TXA_{Gser} | 185 | ~~~~~~~~~~M SSGAAKLNHP APEFDDWALM PNGTFFKVSL SSYKGYVVL |
| AHPC_{Gsub} | 182 | DLVGI~~~ ~~ DLVGI~~~ ~~ DLVGI~~~ ~~ DLVGI~~~ ~~ DLVGI~~~ ~~ |
| AHPC_{Gsub} | 182 | DLVGI~~~ ~~ DLVGI~~~ ~~ DLVGI~~~ ~~ DLVGI~~~ ~~ DLVGI~~~ ~~ |
| AHPC_{Gsub} | 182 | DLVGI~~~ ~~ DLVGI~~~ ~~ DLVGI~~~ ~~ DLVGI~~~ ~~ DLVGI~~~ ~~ |
| AHPC_{Gsub} | 190 | LLKASA~~~ ~~ LLKASA~~~ ~~ LLKASA~~~ ~~ LLKASA~~~ ~~ LLKASA~~~ ~~ |
| AHPC_{Gsub} | 190 | LLKASA~~~ ~~ LLKASA~~~ ~~ LLKASA~~~ ~~ LLKASA~~~ ~~ LLKASA~~~ ~~ |
for the reaction with the oxidant.

The kinetics of tryparedoxin peroxidase have been elucidated in detail (1). The apparent net forward rate constants for the reaction of the reduced enzyme (calculated per subunit) with a variety of hydroperoxides were found to be around $10^7 \text{ M}^{-1} \text{s}^{-1}$. To achieve such rate constants the active site cysteine residue must be highly activated. These rate constants are virtually the same for all substrates tested, with the possible exception of ethyl hydroperoxide. This may be due to the fact that the reaction of the enzyme with the substrate is not strictly a first-order reaction with the substrate, but rather a complex reaction that involves the formation of a mixed-disulfide with the enzyme. The rate constants for the reaction of the enzyme with the substrate are therefore calculated by fitting the data to a first-order reaction model.

The kinetics of tryparedoxin peroxidase have been elucidated in detail (1). The apparent net forward rate constants for the reaction of the reduced enzyme (calculated per subunit) with a variety of hydroperoxides were found to be around $10^7 \text{ M}^{-1} \text{s}^{-1}$. To achieve such rate constants the active site cysteine residue must be highly activated. These rate constants are virtually the same for all substrates tested, with the possible exception of ethyl hydroperoxide. This may be due to the fact that the reaction of the enzyme with the substrate is not strictly a first-order reaction with the substrate, but rather a complex reaction that involves the formation of a mixed-disulfide with the enzyme. The rate constants for the reaction of the enzyme with the substrate are therefore calculated by fitting the data to a first-order reaction model.

The kinetics of tryparedoxin peroxidase have been elucidated in detail (1). The apparent net forward rate constants for the reaction of the reduced enzyme (calculated per subunit) with a variety of hydroperoxides were found to be around $10^7 \text{ M}^{-1} \text{s}^{-1}$. To achieve such rate constants the active site cysteine residue must be highly activated. These rate constants are virtually the same for all substrates tested, with the possible exception of ethyl hydroperoxide. This may be due to the fact that the reaction of the enzyme with the substrate is not strictly a first-order reaction with the substrate, but rather a complex reaction that involves the formation of a mixed-disulfide with the enzyme. The rate constants for the reaction of the enzyme with the substrate are therefore calculated by fitting the data to a first-order reaction model.
Sequence Analysis of the Tryparedoxin Peroxidase Gene from *Crithidia fasciculata* and Its Functional Expression in *Escherichia coli*

Marisa Montemartini, Everson Nogoceke, Mahavir Singh, Peter Steinert, Leopold Flohé and Henryk M. Kalisz

*J. Biol. Chem.* 1998, 273:4864-4871.
doi: 10.1074/jbc.273.9.4864

Access the most updated version of this article at [http://www.jbc.org/content/273/9/4864](http://www.jbc.org/content/273/9/4864)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 32 references, 14 of which can be accessed free at [http://www.jbc.org/content/273/9/4864.full.html#ref-list-1](http://www.jbc.org/content/273/9/4864.full.html#ref-list-1)