Structure of a glutathione peroxidase homologous gene involved in the oxidative stress response in *Chlamydomonas reinhardtii*

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Abstract

The organisation and nucleotide sequence of the single copy glutathione peroxidase homologous gene *gpxh* from *Chlamydomonas reinhardtii* is reported. The *gpxh* gene consists of five exons and four introns, and encodes a predicted protein (GPXH) of 162 amino acids. GPXH belongs to the family of glutathione peroxidase (GPX)-like proteins and showed high homology with the deduced amino acid sequences of *gpx*-related genes from yeast (67–78% similarity) and from plants (60–65% similarity). The GPXH from *C. reinhardtii* differs from the well characterized mammalian cytosolic GPX (GPX1) in that it contains a normal cysteine residue instead of a selenocysteine, that the residues responsible for glutathione binding at the reactive center in GPX1 are not present, and that two amino acid stretches important for the tetramerisation of GPX1 are absent. Northern blot experiments revealed a single 1.3 kb mRNA of which the cellular concentration is elevated strongly upon exposure to chemicals causing oxidative stress. In addition, salt stress did cause a weak increase in mRNA concentration. This indicates that *gpxh* is an oxidative stress responding gene rather than a general stress responsive gene. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Glutathione peroxidase (GPX) catalyses the reduction of hydrogen peroxide (H$_2$O$_2$) or organic hydroperoxides to water or alcohols by reduced glutathione (GSH). Since the discovery of GPX in 1957 [1], it has been a thoroughly investigated protein shown to be important in the defense against oxidative stress [2]. The initially characterized mammalian cytosolic GPX (GPX1) exists as a homotetramer with a subunit mass of 21 kDa [3] and contains a selenocysteine (Secys) residue at the active site. Later, several additional types of mammalian GPX were isolated, including the Se-independent epididymal secretory GPX (GPX5) that was first isolated from mice [4].

GPX-like activities have not only been measured in mammals, but also in yeast, in algae and in higher plants [5]. In some of these cases the enzymes exhibiting the GPX-like activities might have been glutathione S-transferases (GST) that are known to exhibit GPX-activity besides their GST activity. In other cases, GPX rather than GST were found: a GPX with an activity restricted to the reduction of organic peroxides has been isolated from the yeast *Hansenula mrakii* [6], and a GPX that metabolized at least some organic peroxides as well as H$_2$O$_2$ was found in *Chlamydomonas reinhardtii* [7]. The latter has been shown to contain selenium and appears to be of tetrameric structure, like the mammalian GPX1. No other plant GPX-like protein has so far been shown to contain selenium or to have a tetrameric nature.
In order to examine the structure, the function, and the regulation of the enzymes with GPX-like activities, attempts were made to isolate the corresponding genes. Indeed, several gpx-related genes have recently been isolated from bacteria [8,9], from the nematode Brugia pahangi [10], from the helminth Schistosoma mansonii [11], from Plasmodium falciparum [12], from yeast (unpublished, accession numbers P38143, P40581, 36014 (swissprot) AB012395 (EMBL)) and from higher plants [5,13–15]. In contrast to the mammalian GPX1 but like GPX5, all these non vertebrate gpx-related genes, except for the SeCys-encoding gene isolated from S. mansonii, carry a codon for a Cys residue at the putative catalytic site instead of the TGA codon for SeCys. This is a striking difference to GPX1, because selenium atoms are essential for catalysis of GPX1 and the replacement of selenium by sulfur results in a reduction of protein activity by more than 99%. This can be explained by the fact that selenium leads to more nucleophilic power and a lower pK than sulfur. Therefore it reacts much faster with hydroperoxides [16]. For the Cys-(instead of SeCys) encoding gpx-like genes it was unclear, whether they encoded for functional GPXs and what their physiological role was. Further studies have revealed that the Cys-containing GPX-like proteins from Citrus sinensis and B. pahangi exhibit GPX activity towards organic hydroperoxides [17,18], even though this activity is considerably lower than reported for the SeCys-containing GPXs. Thus, alternative roles for Cys-containing GPX-like proteins may exist in vivo. Further functional studies were consistent with an important role of selenium independent GPX-like proteins in the cellular stress response: The GPX-like proteins from N. sylvestris and C. sinensis showed elevated expression under Hg-stress and salt stress respectively [19,20], Escherichia coli cells that express the gpx-like gene of the Citrus plant are more resistant against oxidative stress exerted by paraquat [21], and clones of Neisseria meningitidis with an interrupted gpx-like gene show higher sensitivity towards paraquat [22]. The mammalian Se-independent GPX5 has been shown to have GPX activity and to protect cells against elevated concentrations of H$_2$O$_2$ in the culture medium [23]. In this report, we describe the isolation and characterization of a gpx-related gene from C. reinhardtii. Since C. reinhardtii is accessible to a wide variety of genetic and molecular techniques [24,25], it appeared well suited for studying the physiological role of this gene. The work presented here is a first step in our investigation of the oxidative stress response in C. reinhardtii and shows that gpxh plays a role in the response to oxidative stress.

2. Methods

2.1. Strains and culture conditions

C. reinhardtii strain cw$_{15}$arg$_{7}$mt- (CC-325) was cultured in Sueoka high salt medium HSM or Tris–acetate–phosphate-medium TAP [26]. The cultures were agitated on rotatory shakers (150 rpm) under constant illumination of 40 µmol m$^{-2}$ s$^{-1}$ photosynthetically active radiation at 25°C (standard conditions). Cells were grown on solidified HSM containing 1% agar under a cycle of 14 h illumination (25 µmol m$^{-2}$ s$^{-1}$ photosynthetically active radiation) and 10 h dark at 25°C. Media were supplemented with 50 mg/l arginine, 50 mg/l ampicilline and 1.2 g/l sodium acetate when required.

E. coli DH5α [27] was used for routine cloning experiments and was grown on LB at 37°C. E. coli BL21(DE3)(pLysS) [28] was used for the expression of pET8c derived plasmids [29].

2.2. Nucleic acid preparation

Genomic DNA of exponentially growing cultures of C. reinhardtii cw$_{15}$arg$_{7}$mt- was isolated by lysing cells in 100 mM Tris–HCl pH 8.0, 2% CTAB, 1.4 M NaCl, 20 mM EDTA and 2% b-Mercaptoethanol at 65°C for 2 h, extracting the lysate with phenol, precipitating contaminating polysaccharides in 1% CTAB, 70 mM NaCl, 1 vol. chloroform/isoamyl alcohol, and by a final precipitation of the DNA with isopropanol [30].

Total RNA was prepared by the acid guanidine isothiocyanate–phenol–chloroform method [31] using TRIzol Reagent (Life Technologies Ltd.).

2.3. Construction of cDNA library

Three 100-ml TAP cultures of C. reinhardtii strain cw$_{15}$arg$_{7}$mt- were grown to a density of 3×10$^{6}$ cells per ml. Two of the cultures were then inoculated with 1 mM H$_2$O$_2$ and 0.1 µM paraquat
respectively and grown for an additional 4 h. The three cultures were pooled and poly(A)-containing RNA was isolated using the fast track mRNA isolation kit (Invitrogen).

Custom cDNA library construction and directional cloning into the yeast expression vector pYES2 has been done by Invitrogen. The library consists of 10^6 primary recombinants with a mean insert size of 1 kb.

2.4. Isolation of full length cDNA and genomic DNA of gpxh

Total RNA from C. reinhardtii cw15arg7mt- was employed in a reverse transcriptase reaction (RT) using the ready-to-go T-primed first strand synthesis kit (Pharmacia) according to the manufacturer's directions. The single stranded cDNA served as a template for PCR with a pre-cycle incubation at 94°C for 2 min., and subsequent 35 cycles of 1 min at 94°C, 1 min at 60°C, and 90 s at 72°C, followed by a final incubation at 72°C for 4 min. As primers, the RT-primer (5'-AACTGGAAGAATTCGCGGCCGAGGAAT-3') and a degenerated oligonucleotide (5'-GCMT-TYCCBTGYAAYCRTTYGG-3') derived from a consensus amino acid sequence (AFPCNQFG) in glutathione peroxidases were used. The PCR product was cloned via TA cloning into the pT7blue vector (Novagen) and used as a probe for the isolation of gpxh containing cDNA and genomic DNA from libraries using the colony blotting protocol of Ausubel et al. [30]. For stringent hybridizations, the final wash in 0.1 SSC and 0.1% SDS was carried out at 63°C. For non-stringent hybridizations, the final wash was carried out in 2 × SSC and 0.1% SDS at 60°C. At washing temperatures below 60°C, a strong unspecific background was obtained. A first screening of 500,000 clones of the cDNA library revealed 20 positive plaques. In order to find cDNA clones with a potential complete 5' end, PCR amplification was conducted on positive plaques of the cDNA library with a primer complementary to the multiple cloning site in pYES2 vector upstream from the 5' end of the cloned cDNA insert (5'-CGACTCATAAGGGAATATTAGC-3') and a primer complementary to the coding strand of the cDNA as derived from the RT-PCR product (5'-AATGGGGAACGTAACGCCGAA-3'). PCR amplification conditions were: pre-cycle incubation at 94°C for 2 min, and subsequent 35 cycles of 50 s at 94°C, 1 min at 60°C and 2 min at 72°C, followed by a final incubation at 72°C for 4 min. The colonies from the area of the plaque that revealed the longest PCR product were removed and plated out for a second screening. After a third analogous screening step, a single positive colony, designated pGPXa3, could be isolated and was chosen for further analysis.

In order to obtain genomic DNA containing the gpxh gene, 50,000 clones of a cosmid library, which was kindly provided by Donald. P. Weeks [32] were screened. Cosmid cGPX1.4 was selected out of six positive colonies for further investigation.

2.5. Overexpression of gpxh in E. coli

DNA fragments containing the gpxh coding sequence were cloned into pET8c [29] and introduced into E. coli BL21(DE3)(pLysS) [28]. The transformants were grown at 25°C in LB medium to an OD600 = 0.5, overexpression was induced with 1 mM IPTG and cells grown to OD600 = 1.0. Crude extract was prepared in the presence of 1 mg/l pefabloc sc proteinase inhibitor (Boehringer) using a french press.

2.6. Dynamics of gpxh mRNA

Cultures of C. reinhardtii cw15arg7mt- were grown to a density of 1–4 × 10^6 cells per ml in HSM, exposed to 0.2 mM H_2O_2, 0.2 mM tert-butyldihydroperoxide (t-BOOH), 0.5 μM Paraquat or 50–200 mM NaCl and total RNA was extracted from aliquots taken at regular intervals. Aliquots of 20 μg of total RNA were denatured with glyoxal/DMSO, blotted, hybridized and washed following the protocol of Ausubel et al. [30]. The last wash in 0.1 × SSC and 0.1% SDS was carried out at 63°C. The amount of total RNA was controlled by staining with ethidium bromide.

2.7. Sequencing

Fragments of cosmid clone cGPX1.4 and cDNA clone pGPXa3 were subcloned. The fragments of pGPXa3 were sequenced in both directions whereas the restriction sites joining the different fragments as well as most of the chromosomal
DNA were sequenced only in one direction. Sequencing reactions with IRD41-labelled primers M13universal and M13reverse (MWG-Biotech) were carried out with the labstation thermo sequenase labeled primer cycle sequencing kit (Amersham) following the manufacturer’s instructions and they were analyzed on a LI-COR sequencer 4000 using the Base ImagIR software package (LI-COR). Accession number of the sequence on EMBL and GenBank: AF014927.

2.8. Primer extension

Primer extension was carried out in an optimized reaction mixture with two different IRD41 labeled primers (IRGPX5.1: 5'-GGTGTTAGGC-GATTGGTTATTG-3'; IRGPX5.4: 5' -TCGTCGACAGGCCGTAAAACT-3') (MWG biotech) complementary to the 5' untranslated region of the gpxh mRNA. In a total volume of 14 μl, 20 μg of total RNA, 10 pmol of one primer, 1 μl DMSO and water were incubated at 93°C for 2 min and directly cooled to 42°C. Subsequently, 4 μl of a 5× AMV RT buffer (Amersham), 1 μl dNTP mix (10 mM each) and 0.5 μl AMV RT (5 U) were added and the reaction was performed for 1 h at 42°C. After heating the reaction mixture to 95°C for 5 min, the RNA was digested with 10 ng RNase at 37°C for 30 min. The cDNA was precipitated with ethanol and analyzed on a LI-COR sequencer 4000.

To determine the size of the cDNA, the same primers were applied in a sequencing reaction on the corresponding chromosomal DNA.

3. Results

3.1. Isolation of gpxh genomic DNA and cDNA

A glutathione peroxidase homologous gene (gpxh) has been isolated from C. reinhardtii by reverse genetics. Total RNA extracted from C. reinhardtii was used to generate single stranded cDNA with oligo dT18 as a primer. This cDNA served as a template in a PCR using the dT18 primer in combination with a degenerate primer deduced from conserved amino acid sequences in GPX-like proteins from other organisms. The sequence of the obtained band contained an open reading frame with a deduced amino acid sequence exhibiting high homology to known glutathione peroxidases.

This PCR product was used as a probe to screen a cDNA and a cosmid library of C. reinhardtii. Out of 500 000 screened cDNA clones, 20 clones hybridized with the probe. From these, the cDNA clone pGPXα3 with the longest 5' region as determined by PCR analysis was used for further studies. Among 50 000 cosmid clones, six clones hybridized with the PCR product. In a southern blot analysis, the clone named cGPX1.4 proved to contain the longest gpxh-containing chromosomal fragment and thus was chosen for subcloning and further analysis.

3.2. Gene organization

Southern blot analysis, performed under low stringency conditions, with the full-length cDNA clone as a probe revealed that the C. reinhardtii gpxh is a single copy gene (data not shown). This result was supported by hybridization patterns of cDNA and cosmid libraries under stringent and non-stringent washing conditions. The stringency of the washings did not influence the hybridization pattern. In view of the fact that Yokota et al. [7] have isolated a Se-dependent GPX from C. reinhardtii, which is related to mammalian GPX1, this is an unexplained, remarkable result. Sequence analysis and primer extension experiments indicated that the dominant (see below) 1218 nucleotides long gpxh mRNA contained a 5' untranslated region of 137 nucleotides followed by an open reading frame of 486 nucleotides and a 3' untranslated region of 592 nucleotides. The putative translation start codon is flanked by a purine in position -3 and G in position +4 (AGCATGG) characteristic for strong eucaryotic initiator codons [33]. The 3' end of the mRNA carries a putative polyadenylation signal (TG-TAA) found in other Chlamydomonas genes [26,34] and ends in a poly(A)-tail. Comparison of the cDNA with genomic DNA sequences implied that the gpxh gene is composed of five exons as shown schematically in Fig. 1. The four introns were localized in the open reading frame region of gpxh and their boundaries were verified by the eucaryotic splice junction consensus sequence [35]. As observed for other C. reinhardtii genes, the introns are relatively small ranging from 153 to 288 bp. The GC content of the gpxh mRNA is high (54.8%) but below the average GC content of
other C. reinhardtii nuclear genes of 64.0% [36]. The codon usage of the gpxh reflects the codon usage of C. reinhardtii nuclear genes very well (data not shown).

3.3. Deduced amino acid sequence and comparative analysis

The identified open reading frame (486 bp) encodes a putative protein (GPXH) of 162 amino acids with a predicted molecular mass of 18.0 kDa. No potential signal peptide was found. The deduced amino acid sequence shows a high similarity (67–78%) to the deduced amino acid sequence of the previously sequenced gpx homologous genes of yeast (Fig. 2). It also exhibits significant homology to GPX-like proteins from several plants (between 60 and 65% similarity). Like these yeast and plant GPX-like proteins, C. reinhardtii GPXH differs from the classic GPX1 in that it contains a Cys instead of the SeCys at the catalytic site. Apart from this difference, all GPX-like proteins contain the identical residues in the catalytic triad (Cys or SeCys, Gln, Trp) as well as identical amino acid regions in three protein loops, namely NVA…C (positions 70–75 in Fig. 2), L.FPCNQF…Q (positions 100–110), and WNF (positions 191–193) that are important for the catalytic activity in GPX1 [2].

The calculated similarities of GPXH from C. reinhardtii with human GPX1 and GPX4 are almost equal (60 and 57%, respectively). C. reinhardtii GPXH (like the yeast and plant GPX-like proteins) resembles, however, the mammalian GPX4 more than GPX1 in that both GPXH and GPX4 lack the GSH binding residues identified in GPX1 and that a large loop (LMTDPKL-ITWSPVCR, positions 172–186 in Fig. 2 (human GPX1 sequence)) and a small helix (LNSL, positions 119–122) are absent that have been shown to be important for subunit interaction in GPX1 [2].

3.4. Transcription start site and promoter of gpxh

The transcription start site of C. reinhardtii gpxh was determined in a primer extension experiment with several samples of total RNA extracted from C. reinhardtii (Fig. 3). Three subsequent nucleotides (TGC) which are located 139–137 nucleotides upstream of the coding sequence could be identified as transcription start sites of gpxh mRNA, the C residue being the most prominent. A smaller fraction of the mRNAs starts with G and even less mRNA is synthesized starting with T. The primer extension was carried out with several samples of total RNA known to contain different relative amounts of the gpxh mRNA (Northern blot analysis, see below). Since the in-

![Fig. 1. Organization of gpxh gene and mRNA of C. reinhardtii. Numbers indicate the position of the site relative to the transcription start site. Boxed regions are the coding region (dark background) and introns (white background) within the coding region. Thick lines are exons, thin lines the chromosomal DNA upstream and downstream the transcribed region of the gpxh gene. Arrows indicate the putative promoter, the transcription start and the putative translation start of the gene.](image-url)
Fig. 2. Alignment of *C. reinhardtii* GPXH amino acid sequence with the amino acid sequences of several other enzymes of the glutathione peroxidase family (*Saccharomyces cerevisiae*: unpublished, Acc. No. P38143 (swissprot); *Citrus sinensis*: [20]; *Arabidopsis thaliana* plastid-targeted GPX: unpublished, Acc. No. AJ000469 (swissprot); *Nicotiana sylvestris*: [19]; human GPX4: unpublished, Acc. No. X71973; mouse GPX5: [4]; human GPX1: [45]; nematode GPX: [10]; *Escherichia coli* GPXH-like gene: [9]). The boxed sequences are: the catalytic triad (C or X, Q, and W; X indicates selenocysteine), glutathione binding residues in the human GPX1 (R and K) and signal peptides. Highlighted are amino acids homologous to *C. reinhardtii* GPXH.

Upstream from the transcription start site, a putative promoter region could be identified. This region contains the following elements that are common to eucaryotic RNA polymerase II promoters and that are located at the characteristic distances from the transcription start site in the *gpxh* gene: A TATA-box (−29 to −25 from the
most prominent transcription start), two CAAT boxes (−47 to −44 and −77 to −74) and a GC-box (−93 to −88) (Fig. 3).

3.5. Overexpression of gpxh in E. coli

In order to assign an enzymatic activity or a physiological role to GPX, gpxh was overexpressed in E. coli BL21(DE3)(pLysS) to ~10% of the total cellular protein content (data not shown). Peroxidase activity was assayed in crude extracts using glutathione as electron donor and H₂O₂, t-BOOH, or cumene hydroperoxide as electron acceptors, basically following the protocol of the NADPH coupled assay described by Wendel [37]. Commercially available GPX was used as positive control. Unfortunately, no significant peroxidase activity could be measured. As an alternative approach, we checked whether E. coli cells overexpressing gpxh had an increased resistance towards oxidative stress conditions. The gpxh overexpressing E. coli cells did not reveal any increased oxidative stress resistance as compared to control cells (data not shown).

Fig. 3. Identification of the gpxh transcription initiation site by primer extension and gpxh putative promoter region. Panel A: Primer extension of gpxh mRNA. Primer extension and sequence were carried out using primer GPX5.4. Different lanes in the primer extension experiments are RNA samples taken from the same culture after exposure to 2 mM H₂O₂ at the following intervals: (a) 0 min; (b) 15 min; (c) 30 min; (d) 60 min; (e) 120 min (see Fig. 4). Panel B: promoter region of the gpxh gene with putative regulatory elements highlighted. These are: a TATA-box in position −25 relative to the strongest transcription start, two CAAT-boxes at −44 and −72 and a GC-box at −86.
3.6. Dynamics of the gpxh expression

In order to get a hint towards the physiological function of the *C. reinhardtii* gpxh gene, the concentration of the gpxh mRNA was monitored in *C. reinhardtii* cells that were exposed to oxidative stress and to osmotic stress. Northern blot analysis with gpxh cDNA as a probe revealed that the gpxh mRNA content increased strongly after the addition of 2 mM H₂O₂ (Fig. 4) and reached a maximum level between 15 and 30 min after addition, being about eight times higher than that of the unexposed cells. Subsequently, the gpxh mRNA level decreased within one hour below the level of unexposed cells. After 6 h, the gpxh mRNA level of exposed cells equaled that of unexposed cells.

In order to test whether the gpxh mRNA levels increased only upon oxidative stress or whether they increased due to a more general stress response, *C. reinhardtii* cells were treated with sublethal doses of compounds that are known to cause oxidative stress (i.e. 0.2 mM t-BOOH, and 0.5 μM paraquat), or with 200 mM NaCl that causes osmotic stress (Fig. 5). During exposure, cell numbers of the cultures did not significantly change, excluding an acute killing of the cells by the compounds. The agents exerting oxidative stress all lead to a strong accumulation of gpxh mRNA after 30 min and 3 h, whereas salt stress did only result in a small change in gpxh mRNA concentration within the time analyzed. This slight induction is in good agreement with the fact that also salt stress leads to the generation of peroxides, to oxidative stress and increased levels of oxidative stress defensive enzymes [38,39].

4. Discussion

We report here the identification and characterization of the single copy glutathione peroxidase homologous gene (*gpxh*) from *C. reinhardtii*.

The level of gpxh mRNA raised upon exposure of exponentially growing *C. reinhardtii* to chemicals known to cause oxidative stress, like hydrogen peroxide, t-BOOH and paraquat [40]. Also salt stress lead to small changes in the gpxh mRNA level within 3 h. It has been shown previously that the gpx-related gene from *Citrus sinensis* is strongly induced by salt stress. This finding has been explained by the fact that environmental...
stresses like drought and salt stress generate oxidative stress and indeed in many organisms lead to increased levels of mRNAs coding for enzymes involved in the defense against oxidative stress [5]. Our data that show a comparatively fast increase in the gpxh mRNA level upon exposure to H$_2$O$_2$ as compared to induction by NaCl are in good accordance with a model in which the response of plant GPX like proteins upon salt stress is mediated by reactive oxygen species [38].

The mRNA of gpxh from C. reinhardtii contains an open reading frame that codes for a putative GPX-like protein (GPXH) with a predicted molecular mass of 18.0 kDa which showed high homology to GPX-like proteins from yeast, plants and shared characteristic features with the mammalian SeCys dependent phospholipid hydroperoxide glutathione peroxidase (GPX4). GPXH as well as the yeast and plant GPX-like proteins contain a Cys residue at their putative active site in contrast to the well characterized mammalian GPX1 and GPX4 that contain a SeCys residue at their active site. The sulfur atom from Cys in principle is able to catalyze the same reactions as the selenium from SeCys, as a less powerful catalyst.

It is striking that C. reinhardtii GPXH, the plant and yeast GPX-like proteins as well as the mammalian GPX4 lack the same amino acid stretches that are present in GPX1 (Fig. 2). In the case of the mammalian GPX4, the deletions disrupt the interfaces responsible for dimerization and tetramerisation in GPX1 [2], which is in good accordance with the monomeric appearance of GPX4 and indicates that the GPX-like proteins isolated from yeast, plants and GPXH from C. reinhardtii probably occur in a monomeric form as well. Interestingly, the mammalian secretory Se-independent GPX5 do not lack the respective stretches and indeed occur as tetramers [41,42]. An additional effect of the absence of these stretches of amino acids is that the active site is more freely accessible. This is consistent with the ability of GPX4 to attack more complex hydroperoxy lipids and with the weak activity of the GPX-like protein of C. sinensis towards phosphatidylcholine hydroperoxide, whilst no activity towards hydrogen peroxide was measurable [17]. Similarly, weak GPX activities have been measured for the Se-independent GPX like proteins from pig and Brugia pahangi [18,42]. However, Okamura et al. [42] found the GPX activity of GPX5 almost negligible in the porcine epididymal fluid and proposed its physiological role to be rather binding to organic hydroperoxides than metabolizing them.

Our attempts to assign any GPX activity to GPXH expressed in E. coli failed. Possibly, the expression in E. coli does not lead to a functional protein, even though E. coli does contain a GPX-like protein itself [9]. Alternatively, GPXH activity might be restricted to more complex acceptor or donor substrates like different organic hydroperoxides or large thiol compounds that are not metabolized by the other antioxidant enzymes present in C. reinhardtii, such as ascorbate peroxidase in the chloroplast, and catalase and a tetrameric selenium-depending GPX in the cytoplasm [7,43]. Furthermore, alternative roles for GPXH must be taken into consideration.

As an alternative function it has been speculated that some members of the GPX family like GPX4 might rather be involved in signal transduction than in the detoxification of hydroperoxides [44]. The flat hydrophobic surface surrounding the catalytic site of GPX4 would allow an interaction with both lipid layers and proteins. Thereby, oxidation equivalents from peroxides could be transferred via the oxidized active site to protein thiol groups.

Although the northern blot analysis indicated a role of GPXH in the defense against oxidative stress, no GPX activity of GPXH overexpressed in E. coli could be measured, nor did the overexpressed protein increase the oxidative stress resistance of E. coli cells. Hence, the physiological role of this protein remains unclear. Even though for the GPX-like protein from C. sinensis, the induction of mRNA upon oxidative stress, its GPX4-like activity and the protection of E. coli overexpressing the protein, clearly indicate that it is involved in the oxidative stress response, its exact role in the cell has neither been elucidated yet. A lot of effort has yet to be made to elucidate many unexplained observations concerning the role of the different Selenium independent GPX-like proteins that occur in organisms from all kingdoms. We believe that C. reinhardtii is a suitable model organism to get more insight into the role of GPX-like proteins, because it contains both Selenium dependent GPX and Selenium independent GPX-like protein, because it is a comparatively simple haploid eucaryotic model and because it is easily accessible to many modern molecular techniques.
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