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Characterization of the Redox Properties of Poplar Glutaredoxin

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ABSTRACT

The presence of glutaredoxins in plants is now well recognized, but their functions and natural substrates remain largely unknown. Recently, a poplar glutaredoxin has been biochemically characterized and several mutants have been engineered in order to explore its reactivity. This work focuses on some physiological functions of the enzyme. According to our findings, the poplar glutaredoxin can serve as an electron donor to the bacterial 3′-phosphoadenylylsulfate reductase as it supports both the catalysis by the enzyme *in vitro* and complements a methionine auxotroph strain of *Escherichia coli*. In addition, poplar glutaredoxin is able to reduce the *Escherichia coli* ribonucleotide reductase 1a (*in vitro* reduction of cytidine diphosphate). Although this glutaredoxin is described as an electron donor to a phloem-located peroxiredoxin, whose function is to detoxify hydroperoxides, we found that it does not directly reduce hydrogen peroxide or other alkyl hydroperoxides as described for yeast and rice glutaredoxins. However, the poplar glutaredoxin may be involved in the response to oxidative stress as its overexpression in *Escherichia coli* resulted in a higher resistance toward hydrogen peroxide, menadione, and *tert*-butyl hydroperoxide. *Antioxid. Redox Signal.* 5, 15–22.

INTRODUCTION

THE THIOL REDOX STATUS OF THE CYTOSOL is maintained by the thioredoxin (Trx) and the glutathione/glutaredoxin (GSH/Grx) systems. Trx and Grx are small (~10–12 kDa), heat-stable disulfide oxidoreductases, with the conserved active site CXXC [typically CP(Y/F)C for Grx and WC(G/P)PC for Trx] (18, 19). These two proteins are constituted by a characteristic succession of β strands and α helices, the β strands being organized into a central pleated β sheet surrounded by α helices, the whole folding pattern known as the Trx/Grx fold. Initial reducing equivalents for both systems are provided by NADPH. In the Trx system, NADPH reduces Trx reductase, which then reduces Trx. Trx reductase is replaced by glutathione reductase and GSH in the Grx system. Grxs can reduce disulfides with a dithiol mechanism using both their active-site cysteine residues (33). An example is the reduction of the disulfide of the *Escherichia coli* ribonucleotide reductase 1a (*E. coli* RNR1a), which forms when the enzyme reduces ribonucleotides to deoxyribonucleotides under aerobic conditions (1). However, Grxs can utilize a monothiol mechanism for the reduction of mixed disulfides between proteins and GSH (protein-S-SG substrates) (26, 33).

Trx and Grx species can reduce both RNR and 3′-phosphoadenylylsulfate (PAPS) via PAPS reductase (23, 33, 43). Other substrates include a plasma glutathione reductase and a poplar type II peroxiredoxin (6, 36). Monothiol Grxs containing only one active-site cysteine are natural catalysts for the deglutathionylation of protein-S-SG substrates and may be specific for the assembly of iron sulfur enzymes (34, 40, 43).

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Sulfhydryls of cysteine residues are particularly exposed to reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) or superoxide anions, which are formed during normal aerobic growth or during oxidative stress (14). Formation of intermolecular disulfides or overoxidation of thiols to sulfenic, sulfinic or sulfonic acids can occur and thus lead to loss of enzymatic activity, aging, pathological disease, and cell death (3, 12). To challenge the overoxidation of thiols, cells possess a large battery of antioxidant enzymes, in which Trx and Grx play key roles. For example, Trx and/or Grx can reduce peroxiredoxins, non-heme peroxidases that catalyze the reduction of hydroperoxides (8, 36). Trx and Grx are also able to reduce protein-S-SG substrates forming under conditions of oxidative stress (9, 20).

Analysis of the transcriptional regulation and overexpression/deletion of different Trx and Grx isoforms under conditions of hydroperoxide stress has highlighted specific roles for particular genes (7, 13, 16, 24, 31). Two recent studies demonstrate that yeast Grx1 and a Grx from rice are able to reduce directly different alkyl hydroperoxides into alcohol, a reaction generally catalyzed by peroxidases (10, 22).

Whereas there are at least three "classical" dithiol Grxs in *E. coli* (CPYC active site), in yeast there are two of these proteins and three atypical ones with one active site cysteine (33, 34, 43). Apparently, there are only two classical Grxs in human (15, 25). In the genome of *Arabidopsis thaliana*, there are at least four classical Grxs and many other Grx-like proteins, but these proteins were not biochemically characterized and thus the understanding of the functions of Grxs in plants is not as advanced as in bacteria, yeast, or mammalian cells. The first plant Grxs were identified in spinach leaves, in the phloem sap of castor bean, and in the aleurone layer of rice (28, 29, 42). Until recently, the substrates of plant Grxs were unknown, whereas the range of substrates for bacterial Grxs includes redox-sensitive transcription factors (2, 11, 41). Plant Grxs possess dehydroascorbate reduction activity, and the Grx from poplar was demonstrated to be an efficient electron donor for a peroxiredoxin involved in the reduction of hydroperoxides (28, 35).

In this work, we describe the capacity for the poplar Grx to reduce *E. coli* RNR1a and PAPS reductase and to restore the growth of methionine auxotroph strains of *E. coli*, suggesting that plant Grxs can perform functions similar to those described in other organisms. Moreover, the involvement of the poplar Grx in the resistance to ROS was investigated by overexpressing the gene in an *E. coli* BL21(DE3) strain submitted to oxidative stress and by measuring the glutathione peroxidase activity of this Grx.

MATERIALS AND METHODS

Chemicals and reagents

NADPH was obtained from Boehringer Mannheim (Germany). Diamide, H_2O_2, tert-butyl hydroperoxide, menadione, dithiothreitol (DTT), glutathione reductase, and GSH were from Sigma (St. Louis, MO, U.S.A.). Isopropyl β-D-thiogalactoside (IPTG), kanamycin, and ampicillin were from Euromedex (France). All the recombinant Grx were purified as described (35).

Cloning in pBAD and E. coli *mutant strains complementation*

A fragment encoding the poplar Grx was inserted between the restriction sites *NcoI* and *XhoI* of the plasmid pBADHisMyc and called pBAD Grx. This plasmid was used to transform the *E. coli* strains DHB4 *trxA*$^-$ *grxA*$^-$, DHB4 *gor*$^-$ *trxA*$^-$, and DHB4 *grxA*$^-$*grxB*$^-$*grxC*$^-$ (32, 44). The same strains transformed with the plasmid without insert were used as controls. Transformed strains were grown on M9 medium, supplied with Leu, Ile, and Met (50 μg/ml) and ampicillin (100 μg/ml). Single colonies were streaked on the same medium lacking Met but supplied with 0.2% arabinose and kept at 37°C for 48 h.

Reduction of cytidine diphosphate (CDP) by E. coli *RNR*

Reduction of CDP by *E. coli* RNR1a was performed essentially as described (17). Reducing power was provided by 4 m*M* GSH, 1 m*M* NADPH, and 6 μg/ml yeast glutathione reductase. All assays were performed in a total volume of 120 μl. The catalytic rates reported represent the mean value of two independent measurements ± 1 SE.

E. coli *PAPS reductase activity measurement*

PAPS reductase activity was measured as described (23) by following the formation of $[^{35}S]SO_3^{2-}$ from [^{35}S]PAPS. The reaction mixture contained 100 m*M* Tris-HCl, pH 8.0, 3.3 n*M* PAPS reductase, 10 m*M* Na_2SO_3, 60—100 μ*M* [^{35}S]PAPS (specific radioactivity: 1,700 Bq/nmol), and various amounts (0.5–70 μ*M*) of the wild-type (WT) poplar Grx and of an active-site mutant Grx Y29P, mimicking the active site of some plant Trxs. The Grxs were kept reduced by 5 m*M* GSH and 5 m*M* DTT.

In vitro *peroxidase activity of Grx*

The peroxidase activity of poplar Grx was followed using two different spectrophotometric measurements with a Cary 50 spectrophotometer. Firstly, the consumption of H_2O_2 was evaluated in a coupled reaction by following the oxidation of NADPH at 340 nm in the presence of the GSH/Grx system at 30°C. A reaction mixture of 500 μl contained 50 m*M* Tris-HCl, pH 8.0, 2 m*M* EDTA, 150 μ*M* NADPH, 300 μ*M* GSH, 0.5 units of yeast glutathione reductase, and various concentrations of either WT Grx or of the two monocysteinic mutants C27S and C30S. It was incubated for 1 min before the reaction was started by addition of 250 μ*M* H_2O_2. In the second test, the measurement of H_2O_2 consumption was estimated using the Peroxoquant Quantitative Peroxide Assay from Pierce. A 50 μl reaction mixture comprising 30 m*M* Tris-HCl, pH 8.0, 500 μ*M* DTT, 500 μ*M* H_2O_2, and various Grx concentrations (1–50 μ*M*) was incubated for 4–10 min at ambient temperature. An aliquot of 5 μl was then added to the working reagent, and the absorbancy was measured 20 min later at 560 nm.

Resistance of a Grx overexpressing E. coli *strain to oxidants*

The *E. coli* strain BL21(DE3), transformed with the helper plasmid pSBET, was cotransformed with the different

recombinant pET plasmids (37). Single colonies, overexpressing the recombinant proteins, were streaked on Luria Bertani agar plates in the presence of 50 µg/ml ampicillin and kanamycin, 100 µ*M* IPTG, and various concentrations of the oxidants. The cells were allowed to grow for 48 h at 37°C.

RESULTS

Sequence analysis

Grxs are ubiquitous proteins with a highly conserved structural fold, but the amino acid sequences are very divergent in different organisms. Phylogenetic analyses demonstrate that the plant Grx studied here belongs to the plant group, itself related to yeast and fungal Grxs (Fig. 1). The dendrogram shows clearly that those two groups are separated from the archae, the eubacteria, and the mammalian ones. A sixth group is constituted by the 1-Cys Grx, well characterized in yeast. A comparison of selected Grxs relevant to this work is shown in Fig. 2. The closest protein is the *Arabidopsis* Grxs (66% identity), followed distantly by rice and yeast Grx1 (39% identity). The *E. coli* Grx1 and human Grx1 exhibit only 30 and 29% identity, respectively. All these sequences possess a classical active site CP(F/Y)C and the amino acid motif involved with GSH binding ($TVPX_9GG$). Nevertheless, the *Arabidopsis* and the poplar Grxs are a bit elongated as they present an N-terminal extension of ~25 amino acids, whose function is unknown and dispensible (37).

Reduction of E. coli *RNR1a*

In order to explore the physiological functions of the plant Grxs, the poplar Grx was tested as a potential reductant for *E. coli* RNR1a, one of the three potential RNR of *E. coli*. In this

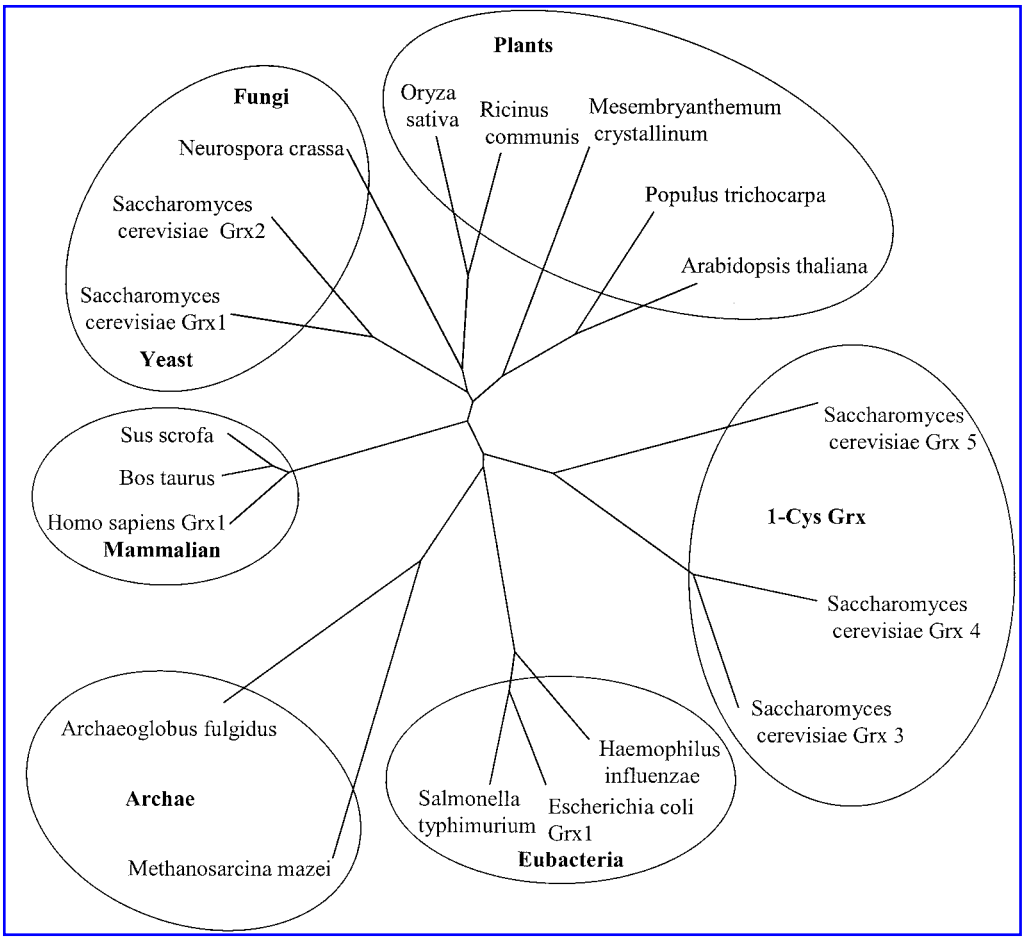

FIG. 1. Dendrogram showing the amino acid sequence relationship between Grxs of different kingdoms. The sequences were aligned with the clustalw software, and the tree was constructed using the Phylip program. Genbank accession numbers are as follows: NP-009895: *Saccharomyces cerevisiae* Grx1; NP-010801: *Saccharomyces cerevisiae* Grx2; NP-010383: *Saccharomyces cerevisiae* Grx3; NP-011101: *Saccharomyces cerevisiae* Grx4; NP-015266: *Saccharomyces cerevisiae* Grx5; BAA20071: *Oryza sativa*; T12219: *Mesembryanthemum crystallinum*; AAL90750: *Populus trichocarpa*; NP-197550: *Arabidopsis thaliana*; S54825: *Ricinus communis*; GDBO: *Bos taurus*; NP-415370: *Escherichia coli*; NP-002055: *Homo sapiens*; GDPG: *Sus scrofa*; I64127: *Haemophilus influenzae*; NP-459849: *Salmonella typhimurium*; P-635295: *Methanosarcina mazei*; NP-070365: *Archaeoglobus fulgidus*; CAB88564: *Neurospora crassa.*

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Populus trichocarpa       MATRIRLPSILATAVTLTVLAASLTWAAGSPEATFVKKTISSHQIVIFSK
Arabidopsis thaliana      -MTMFRSISMVMLLVALVTFISMVSSAASSPEADFVKKTISSHKIVIFSK
Oryza sativa              ------------------------------MALAKAKETVASAPVVVYSK
Saccharomyces cerevisiae  -------------------------MVSQETIKHVKDLIAENEIFVASK
Homo sapiens Grx1         ------------------------------MAQEFVNCKIQPGKVVVFIK
Escherichia coli Grx1     ----------------------------------------MQTVIFGR
                                                                  . : :

Populus trichocarpa       SYCPYCKKAKGVFKELNQTPH----VVELDQREDGHDIQDAMSEIVGR--
Arabidopsis thaliana      SYCPYCKKAKSVFRELDQVPY----VVELDEREDGWSIQTALGEIVGR--
Oryza sativa              SYCPFCVRVKKLFGQLGATFK----AIELDGESDGSELQSALAEWTGQ--
Saccharomyces cerevisiae  TYCPYCHAALNTLFEKLKVPRSKVLVLQLNDMKEGADIQAALYEINGQ--
Homo sapiens Grx1         PTCPYCRRAQEILSQLPIKQG-LLEFVDITATNHTNEIQDYLQQLTGA--
Escherichia coli Grx1     SGCPYCVRAKDLAEKLSNERD--DFQYQYVDIRAEGITKEDLQQKAGKPV
                          . **:*  .    :          :         : : :  *

Populus trichocarpa       RTVPQVFIDGKHIGGSDDTVEAYESGELAKLLGVASEQKDDFKLE---
Arabidopsis thaliana      RTVPQVFINGKHLGGSDDTVDAYESGELAKLLGVSGN-KE-AEL----
Oryza sativa              RTVPNVFINGKHIGGCDDTLALNNEGKLVPLLTEAGAIASSAKTTITA
Saccharomyces cerevisiae  RTVPNIYINGKHIGGNDDLQELRETGELEELLEPILAN----------
Homo sapiens Grx1         RTVPRVFIGKDCIGGCSDLVSLQQSGELLTRLKQIGALQ---------
Escherichia coli Grx1     ETVPQIFVDQQHIGGYTDFAAWVKENLDA------------------
                          .***.:::. . :**  *     : .
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FIG. 2. Alignment of Grx amino acid sequences. Genbank accession numbers are given in Fig. 1. The yeast sequence is Grx1. The active site and the amino acids involved in the binding of glutathione are shaded.

organism, Trx1 and particularly Grx1 seem to be the best hydrogen donors for this enzyme (17, 30). *E. coli* Grx1 served as the positive control. Because of the low K_m of RNR1a for Grx1 (0.13 μ*M*), the reduction of CDP reached a plateau at a concentration of Grx1 at 1 μ*M* (Fig. 3). In comparison, the saturation for the CDP reduction using poplar Grx was not reached even at a 5 μ*M* concentration of enzyme, demonstrating a poor activity with RNR1a. Nevertheless, the activity recorded is clearly above the background lacking Grx.

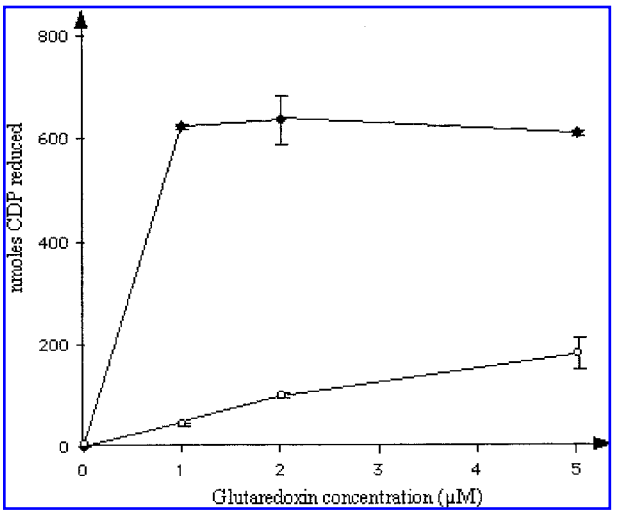

FIG. 3. CDP reduction by *Escherichia coli* RNR. The reduction of CDP by *E. coli* RNR1a was tested in the presence of the GSH system and various concentrations of *E. coli* Grx1 (closed diamonds) or poplar Grx (open circles). The values are the results of two measurements, and the background activity minus Grx was subtracted.

Poplar Grx is an in vitro *and* in vivo *electron donor to* E. coli *PAPS reductase*

Another enzyme that requires Grx for its reduction is PAPS reductase. The capacity of the poplar Grx to serve as an electron donor for this enzyme was investigated in two different ways. First, three *E. coli* strains defective in the Trx and/or Grx systems, unable to grow on minimal medium without Met as a source of sulfate, were functionally complemented with the poplar Grx (Fig. 4). The complementation of all the strains with the plasmid pBAD Grx rescued the growth on minimal medium, whereas the plasmid without insert did not. The observation that the *gor*⁻ mutants can be complemented by Grx is related to their unexpected high content of GSH (44). This suggests that the poplar Grx can reduce PAPS reductase, the key thiol-dependent enzyme involved in sulfate assimilation in bacteria.

To confirm a direct interaction between these two partners, an *in vitro* assay with *E. coli* PAPS reductase was realized (Fig. 5). The Michaelis constants for the reactions utilizing WT Grx and a Y29P mutant that mimics Trx are shown in Table 1. The K_m values of the *E. coli* PAPS reductase for the WT poplar Grx and the Y29P mutant (57.0 and 43.2 μ*M*, respectively) are higher than those for *E. coli* Grx1 (14.9 μ*M*),

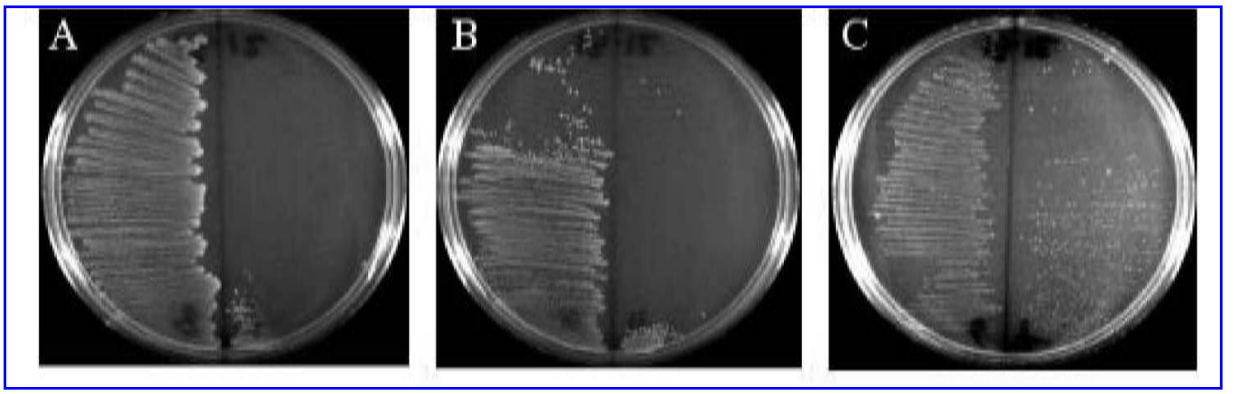

FIG. 4. Growth of DHB4*trxA*⁻*grxA*⁻, DHB4*gor*⁻*trxA*⁻, and DHB4*gor*⁻*grxA*⁻*B*⁻*C*⁻ transformed with pBAD Grx on M9 minimal plates. *E. coli* strains DHB4*trxA*⁻*grxA*⁻ (**A**), DHB4*gor*⁻*trxA*⁻ (**B**), and DHB4*gor*⁻*grxA*²*B*²*C*⁻ (**C**) were transformed with plasmids pBAD Grx (**left**) and pBADHisMyc (**right**) and grown on M9 medium lacking Met as described in Materials and Methods.

one of the three potential reductants of this enzyme along with Trx1 and Trx2. The rate of sulfite formation is a bit higher for the WT poplar Grx (9.6 μmol/mg/min) than for the *E coli* Grx1 (5.1 μmol/mg/min) and for the Y29P mutant (2.6 μmol/mg/min). Finally, the catalytic efficiency of *E. coli* Grx1 is superior to the two poplar Grxs.

Antioxidative properties of poplar Grx

Yeast Grx1 and a Grx from rice have glutathione peroxidase activity toward H_2O_2 and other alkyl hydroperoxides (10, 22). The ability of the poplar Grx and of the C30S mutant to reduce $H_2O_2$and *tert*-butyl hydroperoxide was investigated by following the oxidation of NADPH in the presence of the glutathione/glutathione reductase system. At 300 μ*M* GSH, a slight reduction of H_2O_2 was observed, but there was no further enhancement by adding Grx at concentrations ranging from 1 to 100 μ*M* (data not shown). To confirm that the poplar Grx was active, type II poplar peroxiredoxin was added directly to the reaction medium, resulting in a consumption of NADPH as described (36). The direct reduction of H_2O_2 by glutathione could be bypassed in a second assay where DTT is the reductant and the consumption of $H_2O_2$is measured directly by a colorimetric assay. Varying the concentration of Grx from 1 to 50 μ*M* did not promote any reduction of H_2O_2either (data not shown).

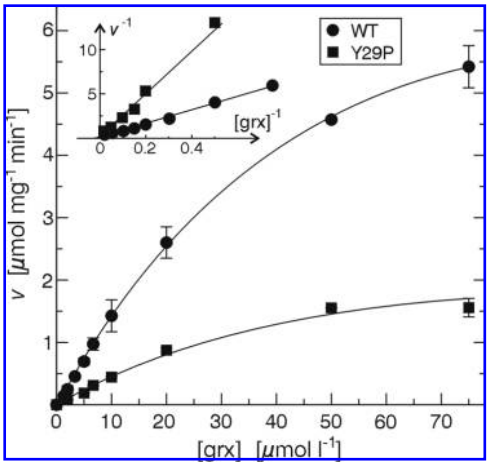

FIG. 5. WT and Y29P mutant of poplar Grx as electron donors for PAPS reductase *in vitro*. Michaelis–Menten and Lineweaver–Burk (**inset**) plots of *V* versus [Grx] are shown. The measurements were done as described in the Materials and Methods. Each point is derived from duplicates of three independent experiments. The kinetic constants were calculated using both nonlinear and linear curve fitting of the data sets.

TABLE 1. KINETIC CONSTANTS FOR GRX IN THE PAPS REDUCTION

	K_m (μ*M*)	V_{max} (μmol/mg/min)	$K_{cat}K_m^{-1}$ (M^{-1} s^{-1})
Poplar WT	57.0	9.6	8.42×10^4
Poplar Y29P	43.2	2.6	3.01×10^4
E. coli Grx1	14.9	5.1	1.71×10^5

As a conclusion, poplar Grx is not directly involved in the reduction of H_2O_2 and other alkyl hydroperoxides. The hypothesis of an indirect effect was investigated by testing the resistance of the *E. coli* strain BL21(DE3) transformed with pET-3d encoding WT Grx and mutants C27S and C30S, in the presence of various oxidants. Transformants with pET-3d Grx grew well in the presence of 1 m*M* H_2O_2, 250 μ*M* *tert*-butyl hydroperoxide, and 500 μ*M* menadione, whereas transformants bearing the control plasmid (pET-3d) or pET-3d Grx C27S did not grow (Fig. 6). Resistance of BL21(DE3)-pET-3d Grx C30S and BL21(DE3)-pET-3d WT Grx was similar for H_2O_2, but not for *tert*-butyl hydroperoxide or menadione. Therefore, poplar Grx can act as a monothiol protein in response to H_2O_2, but only as a dithiol protein in response to *tert*-butyl hydroperoxide and menadione.

DISCUSSION

Poplar Grx reduces *E. coli* RNR1a, but at very low rate compared with *E. coli* Grx1, the preferential hydrogen donor. This low activity of the poplar Grx is not unexpected, because Grxs are species-specific for the reduction of RNR (27). Although the activity obtained with the poplar Grx is low, it

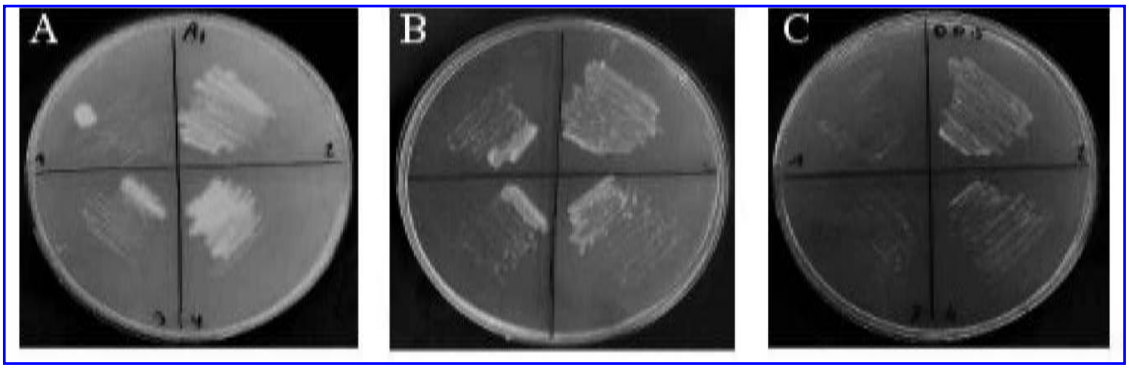

FIG. 6. Growth of *Escherichia coli* BL21(DE3) overexpressing poplar Grx on Luria Bertani plates in the presence of oxidants. Upper left: pET-3d alone; **upper right:** pET-3d Grx; **lower left:** pET-3d Grx C27S; **lower right:** pET-3d Grx C30S. (**A**) 1 m*M* H_2O_2 treatment. (**B**) 250 μ*M* *tert*-butyl hydroperoxide. (**C**) 500 μ*M* menadione.

suggests a similar role for this protein in plants. It would thus be of interest to test this Grx and other similar proteins from poplar in a homologous system together with the RNR of the same source. Actually, only few biochemical data are available about plant RNR. The only enzyme biochemically characterized is a type 1a RNR from *Arabidopsis thaliana,* which *E. coli* Trx and two different cytosolic Trxs from *Arabidopsis thaliana* could reduce. No Grx was tested, however, in these assays (38).

The assimilation of inorganic sulfate into sulfide in many bacteria, including *E. coli,* occurs via a PAPS reductase (23). Recently, a diverse group of sulfate-assimilating bacteria was shown to use 5′-adenylylsulfate (APS) instead of PAPS as a substrate for sulfate assimilation in a pathway analogous to higher plants (5). The *E. coli* PAPS reductase was shown to accept electrons provided by Trx1, Trx2, and Grx1, but not by Grx2 and Grx3, suggesting that there is a specificity between the different Grxs and the PAPS reductase in *E. coli* (23). The poplar Grx was shown here to reduce the PAPS reductase *in vitro* with a lower catalytic efficiency than *E. coli* Grx1. As sulfate assimilation is believed to occur mainly through APS reduction in plants, the physiological significance of this finding needs to be carefully evaluated. As discussed earlier, APS reductases possess, in addition to the domain homologous to the bacterial PAPS reductase, a C-terminal domain possessing a Grx activity (4). *In vitro*, GSH is an efficient hydrogen donor to APS reductase, suggesting that it reduces the Grx domain, which in turn reduces the APS reductase moiety. Nevertheless, the earlier purification of a PAPS-dependent reductase from spinach, together with the discovery of an alternative PAPS gene in knockout APS reductase mutants of *Physcomitrella patens*, raises the possibility of the existence of an alternative PAPS-dependent pathway in higher plants (21, 39). In this context, the capacity of the poplar Grx to reduce plant PAPS reductase could indeed be of physiological importance. The capacity for the poplar Grx to reduce the *E. coli* PAPS reductase was confirmed by complementation of three methionine auxotroph *E. coli* strains deficient in the Trx and Grx pathways. The introduction of the poplar Grx gene restored the growth of each strain in the absence of methionine, confirming that the poplar Grx is indeed able to serve as an electron donor to the PAPS reductase *in vivo.*

We investigated the antioxidative properties of poplar Grx as many studies have demonstrated the involvement of Grxs in the response to oxidative stress. Overexpression of the genes encoding Grx1 and Grx2 in yeast leads to increased resistance to H_2O_2 and menadione, whereas mutant strains lacking these genes were sensitive to H_2O_2 and the superoxide anion forming upon menadione treatment (24). In *E. coli*, lack of Grx activity has been related to increased carbonylation of intracellular proteins and sensitivity to H_2O_2 (17). Yeast Grx1 and a rice Grx possess a high *in vitro* glutathione peroxidase activity (10, 22). This finding was unexpected for yeast as the organism has at least three phospholipid hydroperoxide reductases and five thiol peroxidases (7). We did not detect such an activity for poplar Grx *in vitro*, but its overexpression in *E. coli* increased resistance to H_2O_2, *tert*-butyl hydroperoxide, and menadione, suggesting an indirect role for this Grx. Poplar Grx is a good electron donor to a poplar type II peroxiredoxin and is also able to reduce dehydroascorbate into ascorbate, which is a common antioxidant found in many organisms and a reductant of ascorbate peroxidases (36). Whether poplar Grx is able to reduce the *E. coli* alkyl hydroperoxide reductases, periplasmic thiol peroxidase, or bacterio-comigratory protein is not known. Other potential roles for Grx are protein glutathiolation processes or reduction/oxidation of the transcription factor OxyR, which orchestrates the transcription of many antioxidant proteins in *E. coli* (7).

Perspectives

Based on the data obtained in other organisms, plant Grxs could be involved in the reduction of selected target enzymes, transcription factors, or alternatively in the regulation of several proteins by reversible glutathionylation. These functions are not yet documented in plants. The possibility of regulation of transcriptional factor activity by Grx provides a means for the involvement of these proteins in the transduction of cellular signals or in the phenomenon of glutathionylation, very important in the response to oxidative stress. Interestingly, it is also likely that Grxs could participate in the long-distance signal transduction in plants, because they have been reported as one of the major protein species of the phloem

sap together with Trx (42). Given the sensitivity of this tissue to pathogen attacks, it will be of interest to test whether Grx and Trx are also involved in pathogen-resistance mechanisms.

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ABBREVIATIONS

APS, 5′-adenylylsulfate; CDP, cytidine diphosphate; DTT, dithiothreitol; Grx, glutaredoxin; GSH, reduced glutathione; H_2O_2, hydrogen peroxide; IPTG, isopropyl ß-D-thiogalactopyranoside; PAPS, 3′-phosphoadenylylsulfate; RNR, ribonucleotide reductase; ROS, reactive oxygen species; Trx, thioredoxin; WT, wild type.

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