Phosphoglycolate phosphatase is a metabolic proofreading enzyme essential for cellular function in *Plasmodium berghei*

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*Plasmodium falciparum* (Pf) 4-nitrophenylphosphatase has been shown previously to be involved in vitamin B1 metabolism. Here, conducting a BLASTp search, we found that 4-nitrophosphophatase from Pf has significant homology with phosphoglycolate phosphatase (PGP) from mouse, human, and yeast, prompting us to reinvestigate the biochemical properties of the Pf PGP enzyme. Because the recombinant PfPGP enzyme is insoluble, we performed an extended substrate screen and extensive biochemical characterization of the recombinantly expressed and purified homolog from *Plasmodium berghei* (Pb), leading to the identification of 2-phosphoglycolate and 2-phospho-L-lactate as the relevant physiological substrates of PbPGP. 2-Phosphoglycolate is generated during repair of damaged DNA ends, 2-phospho-L-lactate is a product of pyruvate kinase reaction, and both potently inhibit two key glycolytic enzymes, triosephosphate isomerase and phosphofructokinase. Hence, PGP-mediated clearance of these toxic metabolites is vital for cell survival and functioning. Our results differentiate significantly from those in a previous study, wherein the PfPGP enzyme has been inferred to act on 2-phospho-D-lactate and not on the L isomer. Apart from resolving the substrate specificity conflict through direct *in vitro* enzyme assays, we conducted PGP gene knockout studies in *P. berghei*, confirming that this conserved metabolic proofreading enzyme is essential in *Plasmodium*. In summary, our findings establish PbPGP as an essential enzyme for normal physiological function in *P. berghei* and suggest that drugs that specifically inhibit Plasmodium PGP may hold promise for use in anti-malarial therapies.

The haloacid dehalogenase superfamily (HADSF) is a large family of enzymes consisting mainly of phosphatases and phosphotransferases, which are both intracellular and extracellular in nature. These enzymes are characterized by the presence of a core Rossmannoid fold and a cap domain. Studies of HADSF members have mainly focused on identifying their physiological substrates by screening a wide range of metabolites that include sugar phosphates, lipid phosphates, nucleotides, as well as phosphorylated amino acids and co-factors. This approach has helped us understand the physiological relevance of these enzymes in various cellular processes, such as cell wall synthesis, catabolic and anabolic pathways, salvage pathways, signaling pathways, and detoxification. Apart from dephosphorylating metabolites, HADSF members have also been known to dephosphorylate proteins, and such members are characterized by the absence of the cap domain. A large-scale study reported by Huang et al. (5) has identified a HADSF member from *Salmonella enterica* that catalyzes dephosphorylation of more than 100 phosphorylated substrates. This extended substrate specificity is a common observation in HADSF members and often leads to a confounding situation where determining the physiological substrate of such promiscuous enzymes becomes a challenging task.

Recent studies have identified and characterized HADSF members from the apicomplexan parasite *Plasmodium* (4, 10, 13–16). HADSF members from *Plasmodium* have been found to be involved in processes that lead to the development of resistance to the drug fosmidomycin, which inhibits isoprenoid biosynthesis. Also, these enzymes show considerable activity toward nucleotide monophosphates, phosphorylated co-factors, and generic substrates such as p-nitrophenylphosphate (pNPP) and β-glycerophosphate. A HADSF member that was annotated as 4-nitrophosphophatase from *Plasmodium falciparum* (gene ID PF3D7_0715000) was characterized by Knöckel et al. (15) and proposed to be involved in dephosphorylation.

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5 The abbreviations used are: HADSF, haloacid dehalogenase superfamily; pNPP, p-nitrophenyl phosphate; PGP, phosphoglycolate phosphatase, Pf, *Plasmodium falciparum*; Pb, *Plasmodium berghei*; Ni-NTA, nickel-nitrilotri-acetic acid; RFA, regulatable fluorescent affinity; Tricine, N-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine; CPCSEA, Committee for the Purpose of Control and Supervision of Experiments on Animals; TMP, trimethoprim.
ylation of thiamine monophosphate, the precursor of the active form of vitamin B1 (thiamine pyrophosphate). *In vitro* assays of the purified recombinant enzyme showed that this protein displayed similar specific activities toward thiamine monophosphate and other substrates (ADP, ATP, CTP, Glc-6-P, Fru-6-P, and pyridoxal phosphate) (15). An independent BLASTp search conducted by us revealed that this protein sequence has significant homology (28–30%) with phosphoglycolate phosphatase (PGP) from yeast, human and mouse (Fig. 1). The His6-tagged recombinant *P. falciparum* (Pf) 4-nitrophenylphosphatase, when expressed in *Escherichia coli*, was found to be completely insoluble. However, *Plasmodium berghei* (Pb) 4-nitrophenylphosphatase (gene ID PBANKA_1421300) (referred to as PbPGP hereafter), which shares 69.6% identity (Fig. 1B) with its Pf homolog, expressed in the soluble form in *E. coli* and could be purified to homogeneity. Here we report the biochemical characterization and essentiality of PbPGP. An extended substrate screen identified 2-phosphoglycolate and 2-phospho-L-lactate as relevant physiological substrates in addition to the generic substrates pNPP and glycerophosphate. Attempts at gene ablation showed that the PbPGP gene cannot be disrupted in *P. berghei* despite the loci being nonrefractory for genetic recombination. Our findings emphasize the importance of the “metabolic proofreading” process, which involves clearance or modification of toxic cellular metabolites generated as a consequence of error in substrate recognition by enzymes of intermediary metabolism. This process is universal and analogous to the DNA proofreading observed in polymerases and

![Figure 1. Multiple sequence alignment of phosphoglycolate phosphatase protein sequences.](http://www.jbc.org/)

A, Clustal Omega alignment of phosphoglycolate phosphatase from *P. falciparum*, *P. berghei*, *Saccharomyces cerevisiae*, *Mus musculus*, and *Homo sapiens*. Residues of the conserved HAD motifs involved in catalysis are indicated by asterisks. B, percentage identity matrix showing the extent of homology between the sequences.
Our studies of PbPGP establish the essential physiological nature and biochemical function of this conserved cytosolic enzyme and suggest that drugs that specifically inhibit parasite phosphoglycolate phosphatase can be promising anti-malarial agents.

Results

Biochemical characterization of recombinant PbPGP

BLASTp analysis of Pf and PbPGP protein sequences showed 28–30% sequence homology with phosphoglycolate phosphatase, a conserved protein, present across eukaryotes from yeast to mouse, including humans, involved in metabolic proofreading (Fig. 1A). The Pf and Pb protein sequences show 69.6% identity (Fig. 1B).

Upon expression of C-terminal His₆-tagged PfPGP in the Rosetta DE3 pLysS strain of E. coli, the protein was found to be present completely in the insoluble fraction (Fig. S1A). This was unlike the Strep-tagged PfPGP, which was reported to be present in small quantities in the soluble fraction and, hence, amenable to purification. Therefore, we made use of the protein solubility prediction software PROSOII and found that homologs of PfPGP from other Plasmodium species were predicted to be soluble (Fig. S1B). Hence, the previously uncharacterized P. berghei homolog was chosen for further biochemical studies and physiological investigations. PbPGP was expressed in the E. coli strain Rosetta DE3 pLysS and purified to homogeneity by Ni-NTA affinity chromatography (Fig. S1C), followed by size-exclusion chromatography (Fig. 2A).

PbPGP on analytical gel filtration using a Sephacryl S-200 column showed a mass of about 78 kDa, whereas the theoretical mass is 37 kDa, indicating that the protein is a dimer (Fig. 2, B and C). When further analyzed in the presence of 1 M NaCl, there was a shift in oligomeric state of the protein from dimer toward monomer, suggesting that the oligomers are held by electrostatic interactions (Fig. 2, B and C).

A total of 38 compounds were screened as possible substrates for PbPGP. Although the enzyme displayed very low activity toward nucleotides and sugar phosphates, as reported for PfPGP by Knöckel et al. (15), a novel observation was made as a consequence of our extended substrate screen. PbPGP showed...
P. berghei phosphoglycolate phosphatase

Figure 3. Biochemical and kinetic characterization of PbPGP. A, PbPGP assay performed using 1 mM 2-phospho-D-lactate (DPL) as substrate with and without 10 mM 2-phospho-L-lactate (LPL). The reaction was initiated with 1.89 g of enzyme and incubated for 2 min. B, pH optimum of PbPGP. C, histogram showing the activity of PbPGP in the presence of salts of various divalent cations using pNPP as substrate. D–F, substrate concentration versus specific activity plots fit to the Michaelis–Menten equation for β-glycerophosphate, 2-phospho-L-lactate, and 2-phosphoglycolate. The substrate titration experiment was conducted in two technical replicates containing two biological replicates each. Plots from one technical replicate are shown. Each data point represents mean specific activity value, and error bars represent S.D. (n = 2).

Kinetic studies of PbPGP

PbPGP showed maximum activity at pH 7.0 and preferred Mg$^{2+}$ as a co-factor over other divalent cations (Fig. 3, B and C). The substrate saturation plots for β-glycerophosphate, 2-phosphoglycolate, and 2-phospho-L-lactate were hyperbolic (Fig. 3, D–F) and were fit to the Michaelis–Menten equation to obtain kinetic parameters such as $K_m$ and $V_{max}$ (Table 1). PbPGP has a higher $K_m$ value for 2-phosphoglycolate (3.3- and 11.4-fold) and 2-phospho-L-lactate (27.4- and 6.4-fold) compared with that of murine PGP and yeast Pho13. The $K_{cat}$ value for PbPGP for 2-phosphoglycolate is 11.4- and 3.9-fold higher and for 2-phospho-L-lactate is 37- and 8.9-fold higher compared with that of the murine and yeast homologs, respectively. The catalytic efficiency ($k_{cat}/K_m$) for 2-phosphoglycolate was 3.5-fold higher and 2.9-fold lower compared with its murine and yeast homologs, respectively. With 2-phospho-L-lactate as substrate, the parasite enzyme has a similar catalytic efficiency as its murine and yeast homologs.

Probing the essentiality of PbPGP and localization in P. berghei

The pJAZZ linear knockout vector for PbPGP was generated by following the strategy described by Pfander et al. (18). Drug-resistant parasites were not obtained in the first transfection attempt. In the second attempt, although drug-resistant parasites were obtained, genotyping by PCR revealed nonspecific integration of the marker cassette. These parasites were positive by PCR for both the PbPGP gene and the human dihydrofolate reductase (hDHFR) marker but were negative for specific 5’ and 3’ integration PCRs (Fig. S4). Because it was not possible to obtain knockout parasites, a conditional knockdown (at the protein level) strategy was employed by tagging the gene for PbPGP with a regulatable fluorescent affinity (RFA) tag, where the stability of the fusion protein is conditional to the binding of the small molecule trimethoprim. The conditional knockdown vector was also generated by following the recombiner strategy and validated by PCR (Fig. 4). Transgenic parasites were obtained in the first transfection attempt, and genotyping by PCR showed the presence of a single homogenous population with correct insertion of the RFA tag (Fig. 4F). Nevertheless, it was observed that the reduction in the levels of RFA-tagged protein upon removal of TMP varied between 30–60% across experiments, and complete knockdown could not be achieved (Fig. 5, A–C). As a consequence, there was no significant difference in growth rate between parasites grown in mice fed with or without trimethoprim (Fig. 5, D and E). The transgenic RFA-tagged P. berghei parasites were employed to determine localization of PbPGP, and upon microscopic observation, a cytosolic GFP signal was observed in all intra-erythrocytic stages (Fig. 5F).

Discussion

Earlier, Knöckel et al. (15) performed a TBLASTN search and identified a potential 4-nitrophenylphosphatase in P. falcipa-
The authors proposed a novel role for this HADSF member and suggested involvement in vitamin B1 homeostasis. We found the *P. falciparum* 4-nitrophenylphosphatase sequence to have homology with human, mouse, and yeast phosphoglycolate phosphatases. An extended substrate specificity screen of the recombinant *P. berghei* enzyme revealed that, indeed, this protein is phosphoglycolate phosphatase, which is mainly involved in detoxification, having very high activity on 2-phosphoglycolate and 2-phospho-L-lactate with no activity on thiamine monophosphate. 2-Phosphoglycolate has been reported to be formed during repair of free radical–mediated damage of DNA ends (19), and accumulation of this metabolite in the cell

Table 1

| Kinetic Parameters of *P. berghei* PGP compared with that of homologs from yeast and mouse |
|---------------------------------------------|
| Substrate          | *K*<sub>m</sub> (μM) | *V*<sub>max</sub> (μmol min<sup>-1</sup> mg<sup>-1</sup>) | *k*<sub>cat</sub> (s<sup>-1</sup>) | *K*<sub>m</sub> (M<sup>-1</sup> s<sup>-1</sup>) |
|-------------------|---------------------|---------------------------------|-----------------|-----------------|
| 2-Phosphoglycolate | 2110 ± 11           | 16.2 ± 0.14                     | 10.18 ± 0.08    | 4827            |
| 2-Phospho-L-lactate | 2526 ± 494         | 119.5 ± 12.5                   | 75.15 ± 7.86    | 29,747          |
| 2-Phospho-L-lactate | 4773 ± 574         | 107 ± 13.2                    | 67.34 ± 8.31    | 14,108          |
| 2-Phosphoglycolate | 766 ± 68           | 11.34 ± 0.6                    | 6.56 ± 0.44     | 8564            |
| 2-Phospho-L-lactate | 174 ± 55           | 3.14 ± 0.2                    | 1.82 ± 0.34     | 10,480          |

*<sup>a</sup>* Values taken from Collard et al., 2016 (20).

*<sup>b</sup>* *V*<sub>max</sub> was calculated using *k*<sub>cat</sub> values from Collard et al., 2016 (20). Molecular mass values of 34,540.68 Da and 34,624.58 Da for murine PGP and Pho13, respectively, were used in the calculation.

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**Figure 4.** Generation of the *PbPGP* conditional knockdown construct and parasite. A–D, schematic of *PbPGP* parental, intermediate, and final RFA tagging constructs and *PbPGP* loci after integration. Oligonucleotide primers are indicated by vertical bars, and expected PCR product size is represented by a line between specific primer pairs. E, PCR confirmation of parental, intermediate, and final RFA tagging constructs. F, genotyping of the strain for integration of the cassette in the correct loci. The primer pairs used are mentioned at the top.

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**rum.** The authors proposed a novel role for this HADSF member and suggested involvement in vitamin B1 homeostasis. We found the *P. falciparum* 4-nitrophenylphosphatase sequence to have homology with human, mouse, and yeast phosphoglycolate phosphatases. An extended substrate specificity screen of the recombinant *P. berghei* enzyme revealed that, indeed, this protein is phosphoglycolate phosphatase, which is mainly involved in detoxification, having very high activity on 2-phosphoglycolate and 2-phospho-L-lactate with no activity on thiamine monophosphate. 2-Phosphoglycolate has been reported to be formed during repair of free radical–mediated damage of DNA ends (19), and accumulation of this metabolite in the cell
Figure 5. Phenotypic characterization of PbPGP conditional knockdown parasites and localization of PbPGP. A, Western blot analysis of cell lysates of RFA-tagged parasites from mice fed with trimethoprim (TMP, 30 mg in 100 ml) for 6 days. The experiment was performed twice (EXP1 and EXP2), and a blot from one experimental replicate is shown. Top panel, blot probed with anti-HA antibody, and the arrows indicate the RFA-tagged PbPGP protein. Bottom panel, blot probed with anti-PfHGPRT antibody. B, ratio of the intensity of RFA-tagged PbPGP to the intensity of control HGPRT. C, reduction in PbPGP levels upon removal of TMP relative to the levels in the presence of TMP. Protein levels under the + TMP condition were taken as 100%. D, comparison of growth rates of PbPGP conditional knockdown P. berghei parasites grown in mice fed with TMP or not. The data represents mean ± S.D. values obtained from five mice, each infected with 1.7 × 10⁷ parasites. Statistical analysis was done using a paired t test using GraphPad Prism V5 (p = 0.1560, not significant). E, percentage survival of mice (n = 5) infected with PbPGP RFA-tagged P. berghei parasites and fed with trimethoprim or not. The survival curves were found to be not significantly different according to a log-rank (Mantel–Cox) test (p = 0.2765) and Gehan–Breslow–Wilcoxon test (p = 0.6740). F, localization of PbPGP in PbPGP RFA-tagged parasites grown under the + TMP condition. The erythrocyte boundary is indicated by a white dotted line in the merged panel. DIC, differential interference contrast.
leads to inhibition of the key glycolytic enzyme triosephosphate isomerase (Fig. 6). Studies of phosphoglycolic acid phosphatases from yeast and mouse have demonstrated that this enzyme also performs metabolic proofreading by catabolizing the substrates 2-phospho-D-lactate and 4-phosphoerythronate, which are products of enzymatic side reactions. Activity of PbPGP on 4-phosphoerythronate could not be tested because of nonavailability of the compound. 2-Phospho-L-lactate, generated by phosphorylation of L-lactate by pyruvate kinase, is known to inhibit phosphofructokinase and 4-phosphoerythronate, which is a product of GAPDH side reaction, is known to inhibit 6-phosphoglucuronate dehydrogenase (Fig. 6) (20). Because of the detrimental effect of these metabolites, it becomes essential to clear the cell of these metabolic toxins. This is reflected by the fact that phosphoglycolate phosphatase is an essential gene in the mouse (21). Also, in Arabidopsis, knockout of PGLP1 isomerase leads to impaired post-germination development of primary leaves (22).

Plasmodium, in its intraerythrocytic stages, experiences very high levels of oxidative stress (23), leading to increased reactive oxygen species (ROS) production, which can damage its DNA, the repair of which will result in generation and accumulation of 2-phosphoglycolate. The parasite performs lactic acid fermentation and sequesters large amounts of lactate into the medium, most of which is L-lactate (93–94%) in addition to a small proportion of D-lactate (6–7%), which is known to be produced through the methylglyoxal pathway (24). This lactate can accumulate and be phosphorylated in the cell to give rise to 2-phospholactate. In a recent study, Dumont et al. (16), by metabolite profiling of WT and Δpfpgp P. falciparum, concluded that PfPGP has specificity for 2-phospho-D-lactate, whereas our study contradicts this inference and provides direct evidence for the sole substrate specificity for the L-isomer. In their experiment, Δpfpgp parasites, when grown under normal culture conditions or in the presence of 2 mM L-lactate, showed a similar 12-fold higher accumulation of phospholactate compared with WT parasites grown under the same culture conditions. However, in the presence of increasing concentrations of D-lactate in the culture medium, they observed a dose-dependent increase in the accumulation of phospholactate that was not significantly different between WT and Δpfpgp parasites. Although this rules out the absence of PfPGP activity being the cause for 2-phospho-D-lactate accumulation, the authors have concluded that PfPGP utilizes 2-phospho-D-lactate as a substrate (16). The physiological reasons for these observations of Dumont et al. (16) can be rationalized in the following manner. As L-lactate is the predominant isomer produced in high concentrations in the cell, externally added L-lactate may not be taken up inside the cell or, even when taken up, might not significantly perturb the intracellular L-lactate concentration. Therefore, in the experiment of Dumont et al. (16), addition of L-lactate to the culture medium did not lead to an increase in levels of phospholactate in WT or Δpfpgp parasites. D-lactate is produced in the parasite at very low levels, and exogenously added D-lactate might be acted on by pyruvate kinase to form 2-phospho-D-lactate. This can happen in both
WT and Δpfpgp parasites and the absence of a significant difference in the levels of phospholactate accumulation between WT and Δpfpgp parasites when grown in the presence of D-lactate (16) is expected, as our studies show that PGP is specific for only 2-phospho-L-lactate. Further, Dumont et al. (16) performed metabolite profiling of WT and Δglo1 (impaired in D-lactate production) parasites in the presence of methyl glyoxal in the culture medium and observed similar levels of phospholactate accumulation in both parasites. The authors justify this observation by speculating that either methy glyoxal is converted to D-lactate in erythrocytes and then transported to the parasite or directly converted to D-lactate in the parasite by involvement of the apicoplast glyoxalase-1 (16). Either way, D-lactate levels in the parasite increase, and it is phosphorylated and accumulates in both WT and Δglo1 parasites as phospholactate, in spite of the presence of the PGP gene. This again goes to show that PGP does not act on the D-isomer. As pyruvate kinase is known to have a higher binding affinity for D-lactate compared with that for L-lactate (25), accumulation of phospholactate in WT and Δpfpgp parasites grown on D-lactate, and parasites grown on methyl glyoxal could be a consequence of preferential activity of pyruvate kinase on D-lactate. The indirect inferences provided by Dumont et al. (16) are akin to a “phenocopy” witnessed as a consequence of pyruvate kinase activity on D-lactate rather than a true phenotype associated with phosphoglycolate phosphatase deficiency. Our results regarding the purified enzyme directly show that PbPGP acts only on 2-phospho-L-lactate and not on 2-phospho-D-lactate (Fig. 2D). We further validated this by performing enzyme assays with 1 mm 2-phospho-L-lactate in the presence or absence of 10 mm 2-phospho-D-lactate. The absence of a significant change in specific activity clearly shows that 2-phospho-D-lactate does not bind to the enzyme (Fig. 3A). This observation is consistent with that of the murine homolog of PbPGP, which also acts only on 2-phospho-L-lactate (20). In addition to the evidence above, the possible difference in substrate specificity across the enzymes from the two Plasmodium species, P. falciparum and P. berghei, was also addressed by taking recourse to sequence and structural analysis of the proteins. Both proteins are highly identical, and the residues around the four HAD motifs are highly conserved (Fig. S5A). Both protein sequences were subjected to homology modeling, and both modeled structures aligned without any gross structural differences (Fig. S5, B and C). This strongly suggests that PfPPG, like PbPGP, would also have specificity for only 2-phospho-L-lactate.

The recombinant human pyruvate kinase M2 isoform has been shown to phosphorylate L-lactate, leading to the production of 2-phospho-L-lactate, which, in turn, has been shown to inhibit phosphofructokinase-2 activity in crude lysates of HCT116 cells and activity of the recombinant phosphofructokinase-fructose 1,6-bisphosphatase isozymes PFKFB3 and PFKFB4 (20). Interestingly, in yeast, knockout of PHO13 (a PGP homolog) is viable, as yeast performs alcohol fermentation instead of lactate fermentation and, hence, does not accumulate phospholactate. Also, inhibition of the pentose phosphate pathway caused by accumulation of 4-phospho D-erythronate is countered by transcriptional up-regulation of pentose phosphate pathway enzymes (20). Plasmodium has two genes coding for phosphofructokinase, one on chromosome 9 (PFPK9) and the other on chromosome 11 (PFPK11), and only PFPK9 has been shown to be functional. It has been reported that, unlike the host enzyme, PFPK9 lacks regulation by fructose 1,6-bisphosphate, phosphoenolpyruvate, and citrate (26). In such a scenario, we speculate that 2-phospho-L-lactate might directly inhibit PFPK9 to regulate glycolysis, as knockout of PbPGP is not possible. This selective mode of regulation might be unique to Plasmodium.

In Plasmodium, where glycolysis is the sole source of ATP in asexual stages (27), the parasite cannot afford inhibition of its critical enzymes, such as PKF and triosephosphate isomerase, arising from accumulation of toxic metabolites. Hence, having a metabolic proofreading/detoxifying enzyme becomes vital for its survival. An inability to obtain knockout parasites indicates the essentiality of this protein for parasite survival during asexual stages. To rule out the possibility of the loci being refractory for genetic recombination, RFA tagging was attempted, and a homogenous population of transfectants with the RFA tag integrated at the right loci was obtained. Having established that the locus is amenable for genetic manipulation, a conditional knockdown strategy at the protein level was adopted, making use of the RFA tag (28). Conditional knockdown at the protein level showed only a 30–60% reduction, and, hence, the parasites were viable (Fig. 5, A–E). A similar observation has been described for yoelipain, where the authors were neither able to knockout nor achieve significant knockdown of protein levels to see a growth difference. Therefore, it was concluded that the gene is essential during intraerythrocytic stages (29). Our results are similar and indicate the essentiality of PbPGP in asexual stages. This conclusion regarding the gene essentiality of PbPGP is in agreement with the findings of Dumont et al. (16) regarding PPGP, where Δpfpgp parasites show a growth defect. Further biochemical and structural studies of PGP could pave the way for the rational design of inhibitors with potent anti-malarial activity.

Experimental procedures

Materials

All chemicals, molecular biology reagents, and medium components were from Sigma-Aldrich, New England Biolabs, Gibco, Invitrogen, US Biochemicals, Spectrochem, and Hime-dia. The E. coli strain XL-1 blue, the expression strain Rosetta (DE3) pLysS, and plasmids pET22b and pET23d were from Novagen. The pJAZZ library clone (PbG02_B-53b06); plasmids pSC101BAD, R6K Zeo/pheS, and GW_R6K_GFP-mut3; and E. coli (DE3) pLysS, and plasmids pET22b and pET23d were from Novagen. The pJAZZ library clone (PbG02_B-53b06); plasmids pSC101BAD, R6K Zeo/pheS, and GW_R6K_GFP-mut3; and E. coli (DE3) pLysS, and plasmids pET22b and pET23d were from Novagen. The pJAZZ library clone (PbG02_B-53b06); plasmids pSC101BAD, R6K Zeo/pheS, and GW_R6K_GFP-mut3; and E. coli (DE3) pLysS, and plasmids pET22b and pET23d were from Novagen. The pJAZZ library clone (PbG02_B-53b06); plasmids pSC101BAD, R6K Zeo/pheS, and GW_R6K_GFP-mut3; and E. coli (DE3) pLysS, and plasmids pET22b and pET23d were from Novagen. The pJAZZ library clone (PbG02_B-53b06); plasmids pSC101BAD, R6K Zeo/pheS, and GW_R6K_GFP-mut3; and E. coli (DE3) pLysS, and plasmids pET22b and pET23d were from Novagen. The pJAZZ library clone (PbG02_B-53b06); plasmids pSC101BAD, R6K Zeo/pheS, and GW_R6K_GFP-mut3; and E. coli (DE3) pLysS, and plasmids pET22b and pET23d were from Novagen. The pJAZZ library clone (PbG02_B-53b06); plasmids pSC101BAD, R6K Zeo/pheS, and GW_R6K_GFP-mut3; and E. coli (DE3) pLysS, and plasmids pET22b and pET23d were from Novagen. The pJAZZ library clone (PbG02_B-53b06); plasmids pSC101BAD, R6K Zeo/pheS, and GW_R6K_GFP-mut3; and E. coli (DE3) pLysS, and plasmids pET22b and pET23d were from Novagen.
Bioinformatics analysis

The PfPGP (Plasmodium falciparum gene ID PF3D7_0715000) protein sequence obtained from the PlasmodiumDB database was subjected to a homology search against the nonredundant database at the NCBI using the BLASTp algorithm. Clustal Omega (30) was used to generate multiple sequence alignment. ProSoli (31) was employed to predict the solubility of proteins upon heterologous expression in the E. coli system.

Cloning expression and purification of PfPGP and PbPGP

All expression plasmids with the desired gene of interest were generated in the XL-1 blue strain of E. coli cells. Table S1 lists the oligonucleotide sequences used. The PfPGP gene was amplified by PCR from P. falciparum genomic DNA using the primers P1 and P2. The purified PCR product was digested with the restriction enzymes Ncol and Xhol and ligated with the double-digested plasmid pET23d. Chemically competent E. coli XL-1 blue cells were transformed with the ligation mixture, and transformants were selected on Luria–Bertani medium plates containing ampicillin (100 μg ml⁻¹) and tetracycline (10 μg ml⁻¹). The clones were validated by DNA sequencing. A plasmid isolated from the confirmed clone (pET23d_PfPGP) was used to transform the Rosetta DE3 pLysS strain of E. coli.

A single colony of transformed Rosetta DE3 pLysS was inoculated into 10 ml of terrific broth (TB) medium containing ampicillin (100 μg ml⁻¹) and chloramphenicol (34 μg ml⁻¹) and incubated at 37 °C at 180 rpm for 15 h. The cells were harvested by centrifugation at 18,000 × g for 15 min at 4 °C. The pellets from the centrifuged cell lysate, unbound (flow-through) fraction, were collected. The beads were washed using lysis buffer containing increasing concentrations of imidazole. The collected fractions from Ni-NTA chromatography containing PfPGP were concentrated and subjected to further purification by size-exclusion chromatography on a Sephacryl S-200 column (1.5 × 60 cm).

Determination of the oligomeric state

The oligomeric state of PbPGP was determined by analytical size-exclusion chromatography using a Sephacryl S-200 (1 × 30 cm) column attached to an AKTA Basic HPLC system. The column was equilibrated using 100 mM Tris HCl (pH 7.4) and 100 mM KCl at a 0.8 ml min⁻¹ flow rate and calibrated using the molecular mass standards: β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), BSA (66 kDa), carbonic anhydrase (29 kDa), and cytochrome c (12.4 kDa). 100 μl of PbPGP at 1 mg ml⁻¹ concentration was injected into the column and eluted with equilibration buffer with monitoring at 280 nm. The molecular mass of PbPGP was estimated by interpolating the elution volume on a plot of logarithm of molecular mass standards on the y axis and elution volume on the x axis. Gel filtration was performed with and without NaCl in the equilibration buffer.

Synthesis of 2-phospholactate

Synthesis of both D and L phospholactate was carried out following an available procedure (20). The details of the protocol and characterization of the molecules are provided in Figs. S2 and S3.

Enzyme assays

A comprehensive substrate screen comprising various classes of molecules, such as nucleoside lipases, sugar phosphates, co-enzymes, amino acid phosphates, etc., was performed. The enzyme was carried out in 100 mM Tris HCl (pH 7.4), 2 mM substrate, and 1 mM MgCl₂ in a volume of 100 μl. The reaction mixture was preincubated at 37 °C for 1 min, the assay was initiated using 2 μg of enzyme, and the reaction was allowed to proceed at 37 °C for 5 min. The reaction was stopped by addition of 20 μl of 70% TCA, and 1 ml of freshly prepared Chen’s reagent (water, 6 N sulfuric acid, 2.5% ammonium molybdate, and 10% L-ascorbic acid mixed in the ratio of 2:1:1:1) was added, mixed thoroughly, and incubated at 37 °C for 1.5 h. The color development was measured against a blank (reaction mixture to which enzyme was added after addition of TCA) at 820 nm. Specific activity was calculated using the Δ value of 25,000 M⁻¹ cm⁻¹.

The pH optimum of PbPGP was determined by performing the assay in a mixed buffer containing 50 mM each of glycine, MES, Tris at a different pH level, 1 mM MgCl₂, and 1 mM pNP as substrate in a 100-μl volume. The reaction mixture was preincubated at 37 °C for 1 min; the assay was initiated using 0.2 μg of enzyme, and the reaction was allowed to proceed at 37 °C for 2 min, stopped using TCA, and processed using Chen’s reagent as described above.

The preferred divalent metal ion was identified using 10 mM pNP as substrate and different salts such as MgCl₂, MnCl₂, CaCl₂, CuCl₂, and CoCl₂ at a final concentration of 1 mM in a 250-μl reaction mixture containing 50 mM Tris HCl (pH 8). The reaction was initiated with 0.26 μg of enzyme, and conversion of pNP to p-nitrophenol was continuously monitored at 405 nm at 37 °C. The slope of the initial 20 s of the progress curve was used to calculate specific activity using an ε value of 18,000 M⁻¹ cm⁻¹.

J. Biol. Chem. (2019) 294(13) 4997–5007 5005


**P. berghei phosphoglycerolate phosphatase**

**Kinetic studies**

\( K_m \) values for 2-phosphoglycerolate, 2-phospho-L-lactate, and \( \beta \)-glycerophosphate was determined by measuring the initial velocity at varying substrate concentrations ranging from 0.5–15 mM for 2-phosphoglycerolate and 2-phospho-L-lactate and 0.25–30 mM for \( \beta \)-glycerophosphate. The concentration of MgCl2 was fixed at 5 mM, with the reaction buffer being 200 mM Tricine–NaOH (pH 7.4). The reaction, in a volume of 100 \( \mu \)l, was initiated with 1.89 \( \mu \)g of enzyme, allowed to proceed at 37 °C for 2 min, stopped using TCA, and processed using Chen’s reagent as described above. Specific activity was plotted as a function of substrate concentration, and the data points were fitted to the Michaelis–Menten equation using GraphPad Prism V5 to determine the kinetic parameters (32).

**Generation of P. berghei transfection vectors**

The library clone for *P. berghei* PGP (PbG02_B-53b06) was obtained from PlasmoGem. The procedure for knockout and tagging construct generation has been described previously (33, 18) and is provided in detail in the supporting information.

**Cultivation and transfection of P. berghei**

Male/female BALB/c mice aged 6–8 weeks were used for cultivation and transfection of *P. berghei*. Glycerol stock of WT *P. berghei* ANKA parasites was injected into a healthy male BALB/c mouse. Parasitemia was monitored by microscopic observation of Giemsa-stained smears of blood drawn from a tail snip. Transfection of the parasites was done by following the protocol described by Janse et al. (34), using Amaza 4D Nucleofector (P5 solution and FP167 program) followed by injection into two mice. For PbPGP knockout, drug-resistant parasites were selected by feeding infected mice with pyrimethamine in drinking water (7 mg in 100 ml), whereas parasites with the PbPGP RFA tag were selected by feeding infected mice with drinking water (7 mg in 100 ml), whereas parasites with the PbPGP RFA tag were selected by feeding infected mice with trimethoprim in drinking water (30 mg in 100 ml) and the other with water lacking trimethoprim. Parasitemia was monitored regularly starting from day 2 post-injection by counting parasites in Giemsa-stained smears. The growth rate was determined by plotting the percentage of parasitemia on the y axis against time (number of days) on the x axis. The mortality rate among infected mice fed trimethoprim-containing water or not was also determined by plotting the percentage of survival of mice on the y axis against time (number of days) on the x axis.

**Localization of PGP in P. berghei**

PbPGP RFA-tagged parasites were harvested in heparin solution and centrifuged at 2100 \( \times \) g for 5 min, and the supernatant was discarded. The cells were resuspended in 1 \( \times \) PBS containing Hoechst 33342 (10 \( \mu \)g ml\(^{-1}\)) and incubated at room temperature for 15 min. Thereafter, the cells were collected, washed once with 1 \( \times \) PBS, resuspended in 70% glycerol, and dispersed on poly-L-lysine–coated coverslips that were mounted on glass slides, sealed, and stored at 4 °C. The slides were observed under an oil immersion objective (\( \times 100 \)) of a Zeiss LSM 510 Meta confocal microscope.

**Ethics statement**

Animal experiments involving handling of BALB/c mice were performed by adhering to standard procedures prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), a statutory body under the Prevention of Cruelty to Animals Act of 1960, and the Breeding and Experimentation Rules of 1998, Constitution of India. This study (Project HB006/201/CPCSEA) was approved by the institutional animal ethics committee of the Jawaharlal Nehru Centre for Advanced Scientific Research, which is under the purview of CPCSEA.

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Phosphoglycolate phosphatase is a metabolic proofreading enzyme essential for cellular function in *Plasmodium berghei*

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Correction: Phosphoglycolate phosphatase is a metabolic proofreading enzyme essential for cellular function in *Plasmodium berghei*.

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During copy editing, an error was introduced in a column label in Table 1. The label of the last column should be $k_{cat}/K_m$. This error has now been corrected and does not affect the results and conclusions of this work.

Table 1

| Substrate                  | Kinetic parameters | Kinetic parameters | Kinetic parameters |
|----------------------------|--------------------|--------------------|--------------------|
|                            | $K_m$ (µM)         | $V_{max}$ (µmol min$^{-1}$ mg$^{-1}$) | $k_{cat}$ (s$^{-1}$) | $k_{cat}/K_m$ (M$^{-1}$ s$^{-1}$) |
| **Kinetic parameters of PbPGP** |                    |                    |                    |                                  |
| β-Glycerophosphate         | 2110 ± 11          | 16.2 ± 0.14        | 10.18 ± 0.08       | 4827                             |
| 2-Phosphoglycolate         | 2526 ± 494         | 119.5 ± 12.5       | 75.15 ± 7.86       | 29,747                           |
| 2-Phospho-L-lactate        | 4773 ± 574         | 107 ± 13.2         | 67.34 ± 8.31       | 14,108                           |
| **Kinetic parameters of murine PGP** |                    |                    |                    |                                  |
| 2-Phosphoglycolate         | 766 ± 68           | 11.34$^b$          | 6.56 ± 0.44        | 8564                             |
| 2-Phospho-L-lactate        | 174 ± 55           | 3.14$^b$           | 1.82 ± 0.34        | 10,480                           |
| **Kinetic parameters of *S. cerevisiae* Pho13** |                    |                    |                    |                                  |
| 2-Phosphoglycolate         | 221 ± 13           | 32.87$^b$          | 19.0 ± 0.44        | 85,700                           |
| 2-Phospho-L-lactate        | 747 ± 135          | 13.09$^b$          | 7.57 ± 1.04        | 10,113                           |

a Values taken from Collard et al., 2016 (20).

b $V_{max}$ was calculated using $k_{cat}$ values from Collard et al., 2016 (20). Molecular mass values of 34,540.68 Da and 34,624.58 Da for murine PGP and Pho13, respectively, were used in the calculation.