Trends in
Parasitology

CellPress
OPEN ACCESS

Forum

Protein disulfide isomerases – a way to tackle malaria

Fiona Angrisano [1], Amelia Ford [2], Andrew Michael Blagborough [2,*] and Hayley Elise Bullen [1,*]

Protein disulfide isomerases (PDIs) ensure that specific substrate proteins are correctly folded. PDI activity plays an essential role in malaria transmission. Here we provide an overview of the role of PDIs in malaria-causing *Plasmodium* parasites and outline why PDI inhibition could be a novel way to treat malaria and prevent transmission.

What are PDIs?

The formation and cleavage of disulfide bonds between cysteine residues in proteins is essential for their native conformation and functioning. Underpinning this are the PDIs: multifunctional proteins of the thioredoxin superfamily of redox proteins found in a wide variety of organisms. They play a multifaceted role within cells and maintain three main catalytic activities (thiol-disulfide oxidoreductase, disulfide isomerase, and redox-dependent chaperone) which serve to ensure the correct formation of disulfide bonds [1] (Figure 1). Interestingly, some PDIs in *Giardia lamblia* also exhibit transglutaminase activity *in vitro* [2]. PDIs are characterised by the presence of at least one domain containing a βαβαβαββα thioredoxin-like fold [3] and generally contain four thioredoxin-like domains (abb'a'). The a and a' domains contain the redox active site motif CXXC, while the b' domain contains a large multivalent hydrophobic surface which facilitates structurally promiscuous binding and therefore serves as a chaperone domain [1,3]. Mammalian PDIs are classically considered to be resident primarily in the endoplasmic reticulum (ER) where they facilitate the correct folding and assembly of proteins entering the secretory pathway [1]. They have, however, also been detected in other diverse cellular compartments, including the cytosol, mitochondria, extracellular matrix, and cell surface [4], indicating that they have a much broader role in cell functioning than originally thought. Indeed, they are involved in both the physiology and pathophysiology of various disease states, including neurodegenerative conditions, cancer, cardiovascular disease, parasitic infection, and viral entry into host cells [1,5]. Importantly, they are also found in apicomplexan parasites – which are significant contributors to global morbidity and mortality in human hosts.

The role of PDIs in apicomplexan parasites

The phylum Apicomplexa is a unique and diverse group of parasites capable of causing a variety of diseases in both human and livestock hosts. Over the last decade the important role of PDIs in apicomplexan parasites has become increasingly apparent, with studies finding PDIs at the cell surface of motile and invasive life-cycle stages where they have been implicated in host cell adhesion and invasion, that is, essential processes in their obligate intracellular life cycles [6,7]. Vaccination studies in mice have demonstrated that PDI antibodies can effectively target parasite PDIs. Specifically, mice vaccinated with recombinant TgPDI elicited protection against *Toxoplasma gondii* infection [8], vaccination of mice with recombinant PDI-Trans elicited protection against *Plasmodium berghei* infection [6], whilst vaccination with recombinant NcPDI generated a protective immune response against *Neospora caninum* in mice. The specific PDI substrates underpinning these activities have not been elucidated in either the host or the parasite; however, it has been suggested that parasite PDIs are potentially required for the folding of key proteins involved in parasite invasion, proteins that are either secreted or expressed at the parasite surface.

A key member of the phylum Apicomplexa is *Plasmodium*, the parasite responsible for malaria. *Plasmodium* proteins containing cysteine-rich domains play important roles in many parasite-specific processes, including the invasion of host cells, sequestration in the vertebrate host, and transmission into the mosquito [9]. Although information on *Plasmodium* PDIs is scarce, recent work on a murine model of malaria, involving *P. berghei*, has linked PDI activity to parasite transmission to the mosquito vector [6] – thus implicating *Plasmodium* PDIs as putative novel targets for intervention in transmission life cycle stages.

PDI activity in *Plasmodium* spp.

Plasmodium PDIs are associated with the correct folding of malaria vaccine candidate proteins

PDI activity is currently being extensively studied for its role in various human diseases, yet little is known regarding the expression and function of PDI-like proteins in *Plasmodium* spp. Recent bioinformatic studies have, however, identified nine PDI-like molecules across five species of malaria parasite – four in *P. falciparum* (the species with which the majority of morbidity and mortality is associated), seven in *P. berghei*, and one each in *P. vivax*, *P. knowlesi* and *P. yoelii* – indicated by the presence of classical thioredoxin domains [1,3]. To date, only two of these proteins (PDI-8 and PDI-Trans) have been experimentally proven to have PDI activity [6]. Specifically, detailed analyses of PfPDI-8 (PF3D7_0827900) revealed expression within gametocytes and sporozoites, as well as within the endoplasmic

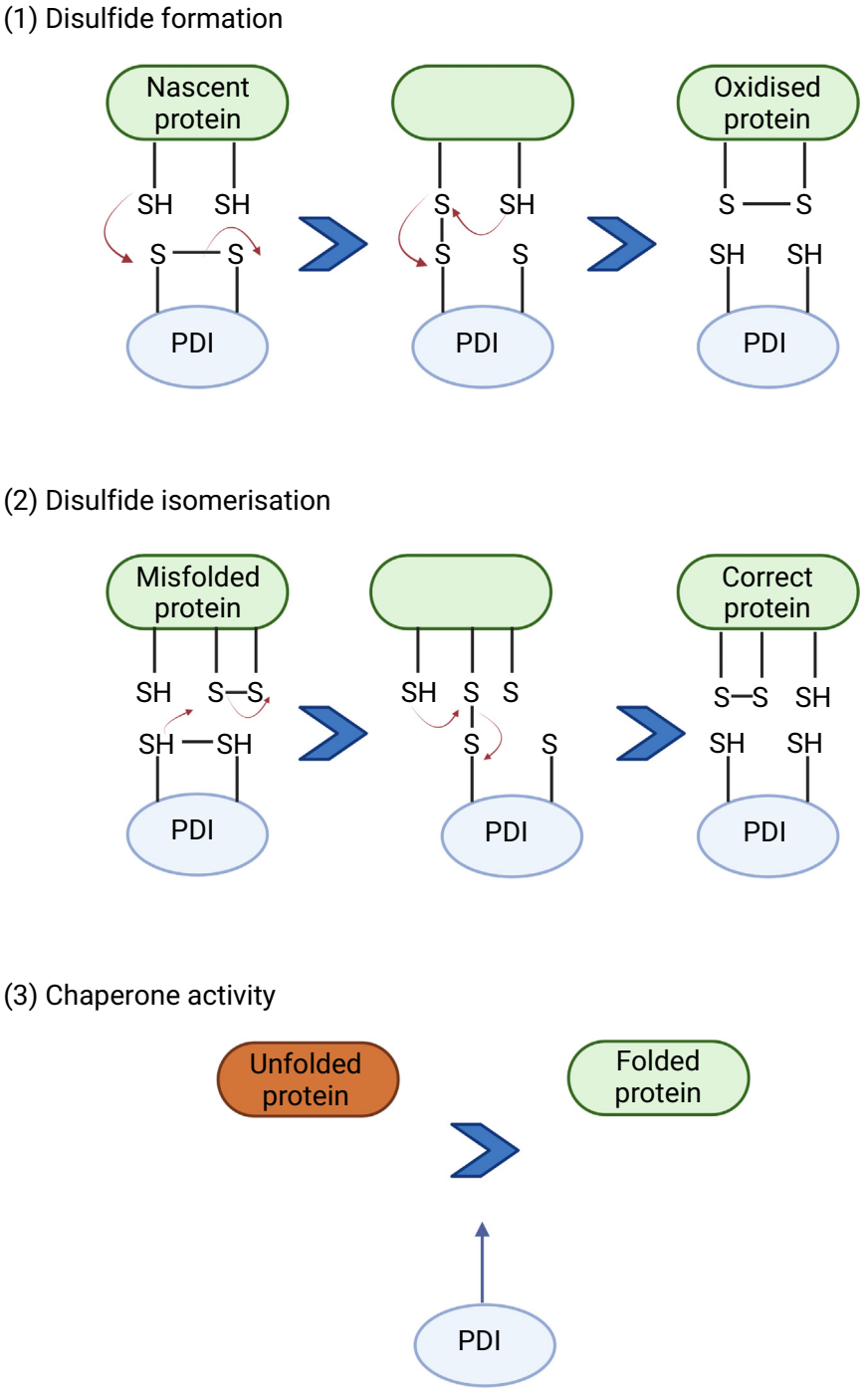

Figure 1. Activities of protein disulfide isomerases (PDIs). PDIs catalyse folding of target proteins by oxidising nascent protein thiols to disulfides (1), rearranging incorrect disulfides (2), and chaperone activity (3). Figure created with BioRender.com. This figure was exported under a paid subscription.

reticulum of asexual schizonts [10]. Biochemical analysis of its role indicated that PfPDI-8 functions in the disulfide-dependent conformational folding of a recombinant form of the erythrocyte-binding protein (and putative blood-stage vaccine target) EBA-175 [11] and could therefore be linked to parasite invasion of red blood cells.

PDI activity is essential for transmission of *Plasmodium* parasites

Recent work has revealed that PDI-Trans (PBANKA_0820300) is essential for transmission of malaria parasites to the mosquito vector in a *P. berghei* mouse model of malaria [6]. PDI-Trans is transcribed and translated across the entire parasitic life cycle and has demonstrated essential activity only in sexual stages when fertilisation of the gamete parasite forms occurs [6] (Figure 2). Furthermore, its disulfide isomerase reductase activity was found to be upregulated post-activation of gametes [6]. Importantly, PDI-Trans is expressed on the surface of male sexual *Plasmodium* parasites, and its activity can be blocked by addition of PDI inhibitor bacitracin. PDI-Trans male gametes display abnormal exflagellation, and knock-out mutants do not progress to the following (ookinete) stages of malarial infection within the mosquito midgut [6], validating PDI-Trans as a potential novel target for antimalarial development. Furthermore, PDI-Trans function is linked exclusively to male sterility, with PDI-Trans exhibiting no observable phenotype on female sterility.

As with most other systems where PDI is implicated in pathogenic activity, the specific mechanisms by which PDI-Trans affects parasite transmission remains unknown. However, successful fertilisation in *Plasmodium* requires the presence and function of a range of proteins with conserved disulfide bonds between cysteine residues on the gamete surface. The 6-Cys family members P48/45, P47, and P230 have all been shown to mediate

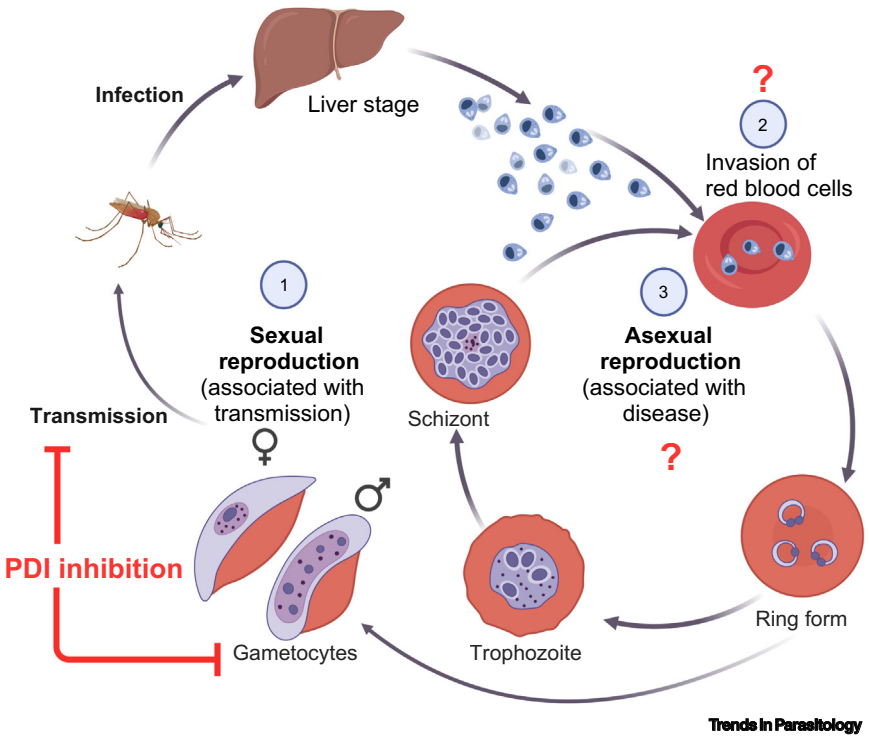

Figure 2. Transmission cycle of *Plasmodium* parasites and stages (1–3) at which protein disulfide isomerase (PDI) activity is postulated to be important. Previous studies have shown that PDI activity is involved in malaria parasite transmission (1) and that this activity can be blocked with commercial PDI inhibitors [6]. PDI inhibition in the related apicomplexan parasite *Toxoplasma gondii* results in invasion inhibition [14]; therefore it is possible that PDI inhibition would also prevent *Plasmodium* spp. invasion (2). Furthermore, the presence of four PDI genes in *P. falciparum* suggests that PDI activity is also required for asexual growth (3), and inhibiting this activity is expected to have a detrimental effect on the parasite. Figure created with BioRender.com. This figure was exported under a paid subscription.

gamete adhesion, whereas the class II fusion protein HAP2 requires the correct formation of multiple crucial disulfide bridges to enable membrane fusion and cytoplasmic continuity [12]. It cannot be discounted that PDIs may, in some way, catalyse disulfide bond rearrangement in one or more of these previously identified transmission-essential proteins, or in yet unidentified substrates, to aid the mediation of gamete adhesion or fertility.

Inhibition of PDI function

To study the function and disruption of PDI-derived activity in a range of *in vitro*, *ex vivo*, and *in vivo* models, chemical inhibitors are commonly used. Inhibitors such as bacitracin, dithiobis nitrobenzoic acid (DNBT), and tocinoic acid have been widely used to study the function of PDIs in diverse cell types.

The most used 'classical' PDI inhibitor is the peptide antibiotic bacitracin [6,7,13]. Bacitracin is produced by some strains of *Bacillus licheniformis* and *Bacillus subtilis*, and in its commercial form is a mixture of at least 22 structurally related peptides, each of which have varying degrees of PDI inhibitory activity (20 μM–1050 μM against purified bovine PDI) [13]. Bacitracin forms bonds with free cysteines in the substrate-binding domain of PDI to inhibit its functioning [13] and has been used to uncover key insights into PDI activity in a variety of apicomplexan parasites, including but not restricted to *T. gondii* and *Plasmodium* spp. [6,7,14]. Specifically, treatment of these parasites with bacitracin resulted in reduced host cell adhesion (*Neospora caninum*), inhibition of parasite invasion (*T. gondii*) [14], and a male-specific inhibition of parasite transmission (*P. berghei*) [6], all consistent with inhibition of the classical function of parasite surface PDIs.

Less commonly used PDI inhibitors include DNBT and tocinoic acid. Like bacitracin, DNBT is membrane impermeant and can be used to inhibit surface proteins on intact cells. DNBT is a sulfhydryl block which can also be used to quantitate thiols in proteins, cells, and plasma, and has been shown to affect host cell entry by viruses such as HIV [15]. Like bacitracin, it has also been found to reduce the ability of *N. caninum* to bind to host cells, therefore reducing its invasion efficiency [7]. Tocinoic acid (a 20-membered cyclic disulfide ring which has the tocin ring of oxytocin) also inhibits host cell binding by *N. caninum* through inhibition of NcPDI [7].

Small-molecule PDI inhibitors (e.g., PACMA31, LOC14, ML359) are now also being developed for the treatment of various mammalian pathologies. Given the broad ranging cell types in which PDIs act, such small-molecule PDI inhibitors should not be overlooked for their potential as repurposed novel treatments for parasitic infections, specifically as novel antimalarial compounds with efficacy across the entire parasitic life cycle. Given the low homology between mammalian and *Plasmodium* spp. PDI proteins, it is likely that – through medicinal chemistry-based refinement – these molecules could be adapted to enhance their specificity to *Plasmodium* PDIs.

Concluding remarks

PDIs are important proteins involved in multiple mammalian disease states, as well as the propagation of multiple viral and parasitic infections. Studies in apicomplexan parasites have demonstrated essential roles for PDI activity in both parasite

invasion and transmission, revealing PDIs as important putative targets for intervention. Further studies are required to determine whether PDI activity is important for *Plasmodium* parasite invasion and asexual growth, which would enable development of dual life-cycle stage inhibitors against malaria. Importantly, further analysis and development of small-molecule PDI inhibitors specific to *Plasmodium* spp. could be highly beneficial in the treatment of *Plasmodium* infections and could prevent not only disease pathogenesis, but also disease transmission which could ultimately aid in malaria elimination.

Acknowledgments

We apologise to colleagues whose primary work was not cited because of space limitations. This work was supported by grants from the Burnet Institute Funding Research Enhancing Data to Accelerate Award (F.A. and H.E.B.) and A.M.B. and A.F. are supported by the MRC [MR/N00227X/1 and MR/W025701/1], Sir Isaac Newton Trust, Alborada Fund, Wellcome Trust ISSF and University of Cambridge JRG Scheme, GHIT, Rosetrees Trust (G109130) and the Royal Society (RGS/R1/201293) (IEC/R3/19302).

Declaration of interests

The authors declare no competing interests.

[1]Burnet Institute, 85 Commercial Road, Victoria, Australia 3004
[2]Division of Microbiology and Parasitology, Department of Pathology, Cambridge University, Tennis Court Road, Cambridge, CB2 1QP, UK

*Correspondence:
amb283@cam.ac.uk (A.M. Blagborough) and hayley.bullen@burnet.edu.au (H.E. Bullen).

https://doi.org/10.1016/j.pt.2023.05.007

References

1. Ali Khan, H. and Mutus, B. (2014) Protein disulfide isomerase a multifunctional protein with multiple physiological roles. *Front. Chem.* 2, 70
2. Davids, B.J. *et al.* (2004) Dependence of *Giardia lamblia* encystation on novel transglutaminase activity. *Mol. Biochem. Parasitol.* 136, 173–180
3. Kozlov, G. *et al.* (2010) A structural overview of the PDI family of proteins. *FEBS J.* 277, 3924–3936
4. Turano, C. *et al.* (2002) Proteins of the PDI family: unpredicted non-ER locations and functions. *J. Cell. Physiol.* 193, 154–163
5. Parakh, S. and Atkin, J.D. (2015) Novel roles for protein disulphide isomerase in disease states: a double edged sword? *Front. Cell Dev Biol.* 3, 30
6. Angrisano, F. *et al.* (2019) Male-specific protein disulphide isomerase function is essential for *Plasmodium* transmission and a vulnerable target for intervention. *Sci. Rep.* 9, 18300
7. Naguleswaran, A. *et al.* (2005) *Neospora caninum* protein disulfide isomerase is involved in tachyzoite–host cell interaction. *Int. J. Parasitol.* 35, 1459–1472
8. Wang, H.L. *et al.* (2013) *Toxoplasma gondii* protein disulfide isomerase (TgPDI) is a novel vaccine candidate against toxoplasmosis. *PLoS One* 8, e70884
9. Lyons, F.M.T. *et al.* (2022) *Plasmodium* 6-cysteine proteins: functional diversity, transmission-blocking antibodies and structural scaffolds. *Front. Cell. Infect. Microbiol.* 12, 945924
10. Cobb, D.W. *et al.* (2021) A redox-active crosslinker reveals an essential and inhibitable oxidative folding network in the endoplasmic reticulum of malaria parasites. *PLoS Pathog.* 17, e1009293
11. Mahajan, B. *et al.* (2006) Protein disulfide isomerase assisted protein folding in malaria parasites. *Int. J. Parasitol.* 36, 1037–1048
12. Angrisano, F. *et al.* (2017) Targeting the conserved fusion loop of HAP2 inhibits the transmission of *Plasmodium berghei* and *falciparum*. *Cell Rep.* 21, 2868–2878
13. Dickerhof, N. *et al.* (2011) Bacitracin inhibits the reductive activity of protein disulfide isomerase by disulfide bond formation with free cysteines in the substrate-binding domain. *FEBS J.* 278, 2034–2043
14. Moncada, D. *et al.* (2016) Role of the 52 KDa thioredoxin protein disulfide isomerase of *Toxoplasma gondii* during infection to human cells. *Exp. Parasitol.* 164, 36–42
15. Ryser, H.J.P. *et al.* (1994) Inhibition of human immunodeficiency virus infection by agents that interfere with thiol-disulfide interchange upon virus–receptor interaction. *Proc. Natl. Acad. Sci. U. S. A.* 91, 4559–4563