

# Mechanism of malarial haem detoxification inhibition by chloroquine

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The haem detoxification pathway of the malaria parasite *Plasmodium falciparum* is a potential biochemical target for drug development. Free haem, released after haemoglobin degradation, is polymerized by the parasite to form haemozoin pigment. *Plasmodium falciparum* histidine-rich protein-2 (Pfhrp-2) has been implicated as the catalytic scaffold for detoxification of haem in the malaria parasite. Previously we have shown that a hexapeptide repeat sequence (Ala-His-His-Ala-Ala-Asp), which appears 33 times in Pfhrp-2, may be the major haem binding site in this protein. The haem binding studies carried out by ourselves indicate that up to 18 equivalents of haem could be bound by this protein with an observed  $K_d$  of 0.94  $\mu$ M. Absorbance spectroscopy provides evidence that chloroquine is capable of

extracting haem bound to Pfhrp-2. This was supported by the  $K_d$  value, of 37 nM, observed for the haem–chloroquine complex. The native PAGE studies reveal that the formation of the haem–Pfhrp-2 complex is disrupted by chloroquine. These results indicate that chloroquine may be acting by inhibiting haem detoxification/binding to Pfhrp-2. Moreover, the higher affinity of chloroquine for haem than Pfhrp-2 suggests a possible mechanism of action for chloroquine; it may remove the haem bound to Pfhrp-2 and form a complex that is toxic to the parasite.

**Key words:** histidine-rich protein, *Plasmodium*, malaria, haemoglobin, haemozoin.

## INTRODUCTION

The malaria parasite is fast becoming resistant to commonly used quinoline antimalarial drugs, resulting in an urgent demand for the development of novel drugs to combat this disease [1]. Quinoline antimalarial drugs have been the drugs of choice for a long time. However, despite being in use for more than half a century, and lots of research, the precise mechanism of action of quinoline antimalarial drugs is still not understood [2]. Several theories have been put forward to describe the antimalarial action of quinoline drugs [1,3]. Most of the blood schizontocidal antimalarial drugs are only active against those stages of the parasite that actively degrade haemoglobin and produce haemozoin pigment [1,3]. The haemozoin pigment is formed by polymerization of the haem that is released following haemoglobin digestion [4,5]. Free haem has been proposed as the receptor for chloroquine by Fitch and colleagues [5]. The enzyme haem polymerase, which catalyses the formation of haemozoin from free haem, was described by Slater and Cerami [6], and Chou and Fitch [7], and chloroquine was reported to inhibit haem polymerization *in vitro* [8]. Several quinoline antimalarial drugs have been reported to inhibit haem polymerization [8–11].

However, the mechanism of haem polymerization and its inhibition is still unclear. Apart from initial reports of the haem polymerase enzyme by Slater and Cerami [6], and Chou and Fitch [7], spontaneous, as well as auto-catalytic, haem polymerization have been proposed [12,13]. Recent publications by ourselves, as well as several other groups, have described the necessity of a biological catalyst for polymerization of haem [14,15]. Recently, *Plasmodium falciparum* histidine-rich protein-2 (Pfhrp-2) has been reported to mediate haemozoin formation, which could be inhibited by chloroquine [16]. A large number of repeats with sequences Ala-His-His-Ala-Ala-Asp or Ala-His-His are present in Pfhrp-2 [16–18]. We have previously shown that hexapeptide repeats Ala-His-His-Ala-Ala-Asp in Pfhrp-2 are the

haem binding sites in this protein [18]. Haem binding to synthetic peptides based on this repetitive sequence was proportional to the number of repeat units in the synthetic peptides. These peptides did not promote haem polymerization. However, native, as well as recombinant Pfhrp-2, promotes haemozoin formation [16,18,19].

Thus Pfhrp-2 has been established as a catalyst for haemozoin formation, and haem metabolism has emerged as a potential drug target in the malaria parasite [20]. The purpose of this study was to characterize the haem binding by Pfhrp-2 and to study the effect of chloroquine on this process. The fact that blocking haem processing appears to be highly toxic to the parasite has led to speculation that inhibiting this pathway might be a promising therapeutic strategy for the treatment of malaria. In the present study we provide evidence for the molecular mechanism of action of malarial haem detoxification inhibition by chloroquine.

## EXPERIMENTAL

### Reagents

Competent *Escherichia coli* BL21(DE3)pLysS cells were obtained from Novagen (Madison, WI, U.S.A.). Haemin, SDS, isopropyl  $\beta$ -D-thiogalactoside (IPTG), PMSF, Luria–Bertani media and all other chemicals were purchased from Sigma (St. Louis, MO, U.S.A.).

### Expression and purification of Pfhrp-2

A plasmid containing the gene encoding Pfhrp-2 in a pET 3d vector (Novagen) was transformed into competent *E. coli* BL21(DE3)pLysS cells [21], and colonies were selected for Pfhrp-2 expression. Expression of Pfhrp-2 was induced by the addition of 0.4 mM IPTG, and protein was purified by metal-chelate chromatography on a Ni<sup>2+</sup>-nitrilotriacetate column using imidazole for elution as previously described [16,19].

Abbreviations used: Pfhrp-2, *Plasmodium falciparum* histidine-rich protein-2; Z-Phe-Arg-AMC, benzyloxycarbonyl-Phe-Arg-aminomethylcoumarin; E-64, *trans*-epoxysuccinyl-L-leucylamido-(4-guanidino)butane; DAB, diaminobenzidine; IPTG, isopropyl  $\beta$ -D-thiogalactoside.

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### Native PAGE analysis of the haem–Pfhrp-2 complex

Complex formation was carried out by adding 5  $\mu$ l of 47  $\mu$ M Pfhrp-2 to an Eppendorf tube containing 1  $\mu$ l of 1 M Tris/HCl buffer (pH 8.0) and 2  $\mu$ l of 2 mM haem. In another tube 2  $\mu$ l of 4 mM chloroquine was added before haem and kept at room temperature for 10 min. After addition of haem to each tube, they were incubated for 10 min at room temperature followed by addition of 5  $\mu$ l of 1  $\times$  PAGE sample buffer [0.125 M Tris/HCl, pH 6.8, containing 20% (v/v) glycerol and 0.1% (w/v) Bromophenol Blue, but without SDS and reducing agent]. From each reaction, 5  $\mu$ l samples were loaded on to a 8% (w/v) polyacrylamide gel [resolving gel buffer, 0.375 M Tris/HCl (pH 8.9); electrode buffer, 0.025 M Tris and 0.192 M glycine (pH 8.3)] and electrophoresis was performed at 100 V for 5 h at 4 °C. Gels were stained for protein using Coomassie Brilliant Blue R-250 or were stained for haem peroxidase using diaminobenzidine (DAB). We used the peroxidase activity of haem to differentiate between Pfhrp-2 and haem–Pfhrp-2 complexes. Haem or haemoproteins were specifically detected after electrophoresis by incubating with DAB and hydrogen peroxide as previously described [22]. In brief, after electrophoresis the gels were washed with water and incubated in a mixture of DAB and sodium acetate [6.3 mM DAB in methanol/0.25 M sodium acetate (pH 5.0), 3:7 (v/v)] for 1 h followed by addition of hydrogen peroxide to a final concentration of 30 mM. Stain was allowed to develop for 30 min, after which gels were washed with 30% (v/v) isopropanol in 0.25 M sodium acetate (pH 5.0) and photographed. All steps of DAB staining were performed in the dark in order to avoid haem degradation.

### Haem binding studies using UV spectroscopy

Haem binding studies were carried out using difference absorption spectroscopy in the Soret region. Spectra were recorded on a Perkin–Elmer Lambda Bio 20 spectrophotometer between 190–700 nm at a speed of 120 nm/min and a slit width of 1 nm. Baselines were routinely subtracted from the spectra. All of the measurements were performed at 25 °C. For the titrations, 20  $\mu$ l of 11.73  $\mu$ M Pfhrp-2 was added to the sample cuvette and haem was added in 1  $\mu$ l increments from stock solutions of 250  $\mu$ M and 1 mM. The amount of haem bound to the protein was measured from the difference spectra by monitoring the change in absorbance at 414 nm, using a millimolar absorption coefficient of 71.2 mM<sup>-1</sup>·cm<sup>-1</sup> for the haem–Pfhrp-2 complex (calculated using a 50:1 ratio of protein/haem; results not shown).

The dissociation constant was calculated by analysing data for multiple ligand-binding sites as described previously [23,24]. Since a significant portion of ligand (haem) is bound to protein (Pfhrp-2), it is not appropriate to use the free haem concentration as the total haem concentration. Using the modified method of Dixon [24] for tight-binding ligands, the dissociation constant  $K_d$  for the haem–Pfhrp-2 complex is given by:

$$K_d = [\text{HP}] / \{([\text{P}_T] - [\text{HP}])([\text{H}_T] - [\text{HP}])\}$$

where  $[\text{H}_T]$  represents the total concentration of haem present (bound and free) and  $[\text{P}_T]$  is the total concentration of protein. The concentration of bound haem  $[\text{HP}]$  was calculated using a millimolar absorption coefficient of 71.2 mM<sup>-1</sup>·cm<sup>-1</sup> for the haem–Pfhrp-2 complex.

### Study of haem–chloroquine interaction

Haem–chloroquine binding was studied using the quenching of chloroquine fluorescence by haem as a measure of binding. Fluorescence emission spectra were recorded on a Perkin–Elmer

LS 50B spectrofluorimeter at 25 °C using 297 nm as the excitation wavelength (slit width of 5 nm) and monitoring emission spectra between 350 to 450 nm (slit width of 10 nm) at a scan speed of 120 nm/min. For the binding titration, 30  $\mu$ l of chloroquine from a 100 nM stock solution was added to 3 ml of buffer (10 mM sodium phosphate, pH 7.4) in a sample cuvette, and haem was added using a 10  $\mu$ l Hamilton syringe in aliquots of 1  $\mu$ l from a 1  $\mu$ M stock solution (freshly prepared before use). Data were analysed by a direct plot of fluorescence intensity against the ratio of haem and chloroquine, as well as by a Stern–Volmer plot, a relationship that describes the effect of quencher on the steady-state fluorescence of the sample. The ratio of fluorescence intensities in the absence and presence of quencher (haem) (described as  $I_{00}$  and  $I_T$  respectively), was plotted against quencher (haem) concentration ( $I_{00}/I_T$  versus haem) to give the Stern–Volmer plot. Binding-site calculations were performed using direct-plot analysis and  $K_d$  values for haem–chloroquine complex formation were obtained from the slope of the linear portion of the Stern–Volmer plot.

### Assay of malaria cysteine protease

Cysteine protease activity of the malaria parasite was assayed as described previously [25,26]. Purified digestive vacuole preparations from malaria parasites were used as a source of cysteine protease activity. Enzyme preparations were incubated with Pfhrp-2, haem and chloroquine in different combinations for 30 min before the start of the assay. Activity was monitored by measuring the cleavage of fluorescent AMC from the specific peptide substrate benzyloxycarbonyl-Phe-Arg-aminomethylcoumarin (Z-Phe-Arg-AMC). An excitation wavelength of 380 nm (slit width of 2.5 nm) and an emission wavelength of 460 nm (slit width of 10 nm) were used for the measurements. A specific inhibitor of cysteine proteases, *trans*-epoxysuccinyl-L-leucylamide-(4-guanidino)butane (E-64), which is known to inhibit malaria cysteine protease activity was used as a control for the validation of the assay. All of the reactions and spectroscopic measurements were performed at 25 °C.

### Protein estimation

Protein concentration was estimated by measuring the absorbance at 205 nm using BSA as a control [27].

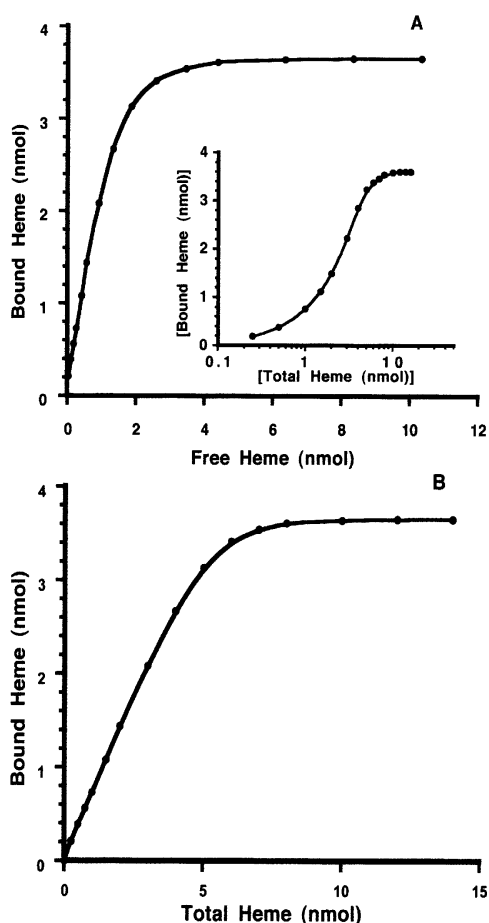
## RESULTS AND DISCUSSION

### Titration of Pfhrp-2 with haem

The haem binding sites of Pfhrp-2 were titrated with increasing amounts of haem (Figure 1). The absorbance at 415 nm increased until it reached a plateau. Data were analysed using the program 'Easybound' [23] made for ligand binding studies based on fitting of data by non-linear regression methods. A  $K_d$  value of 0.94  $\mu$ M for the haem–Pfhrp-2 complex, with 18 haem binding sites, was obtained from the analysis of binding data by non-linear regression analysis. The number of binding sites found by ourselves is in agreement with that reported by Sullivan et al. [16]; however, in another study as much as 50 equivalents of haem were reported to bind to Pfhrp-2 [28].

### Inhibition of haem binding to Pfhrp-2 by chloroquine

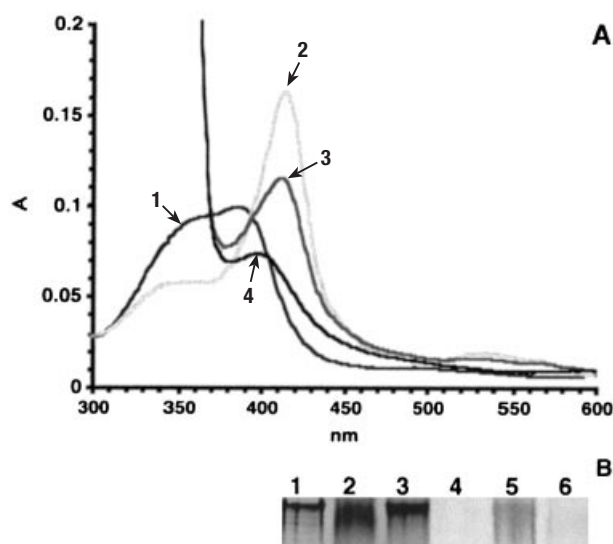
Haem binding to Pfhrp-2 was studied by recording the difference spectra of the protein with and without haem (Figure 2A). Haem alone has an absorption maximum at 385 nm, which is shifted to 415 nm when it binds to Pfhrp-2. When chloroquine was added



**Figure 1** Binding of haem to Pfhrp-2

(A) Bound haem is plotted against free haem for analysis using 'Easybound' software [23]. The inset shows the same data plotted with the  $x$ -axis on a log scale. (B) Direct plot of bound haem against total haem for analysis by the method of Dixon [24]. Details of data analysis are described in the Experimental section. For the titration, 20  $\mu$ l of 11.73  $\mu$ M Pfhrp-2 was added to the sample cuvette and haem was added in 1  $\mu$ l increments from stock solutions of 250  $\mu$ M and 1 mM. The amount of haem bound to the protein was measured from the difference spectra by monitoring the change in absorbance at 414 nm, using a millimolar absorption coefficient of 71.2  $\text{mM}^{-1} \cdot \text{cm}^{-1}$  for the haem–Pfhrp-2 complex.

to the mixture of haem and Pfhrp-2, a significant reduction in haem–Pfhrp-2 complex formation was observed as shown by a fall in the absorption peak at 415 nm. Pre-incubation of chloroquine with haem, before addition to Pfhrp-2, resulted in almost complete loss of haem–Pfhrp-2 complex formation and the spectrum was indistinguishable from that of the haem–chloroquine complex. Removal of haem bound to Pfhrp-2 by chloroquine would interrupt malarial haem detoxification, which would be detrimental to the parasite. Haem–Pfhrp-2 interaction and the effect of chloroquine were confirmed by native PAGE analysis of complex formation (Figure 2B). Lanes 1–3 are stained with Coomassie Brilliant Blue, which detects the Pfhrp-2 protein. Movement of Pfhrp-2 alone (lane 1) was different from a mixture of haem and Pfhrp-2 (lane 2). When chloroquine was added to the reaction mixture along with haem (lane 3), the movement of Pfhrp-2 reverted back to normal. Haem runs ahead along with dye front during electrophoresis. To confirm the presence of haem–Pfhrp-2 complexes, we utilized peroxidase staining using DAB as the substrate (lanes 4–6). DAB specific-



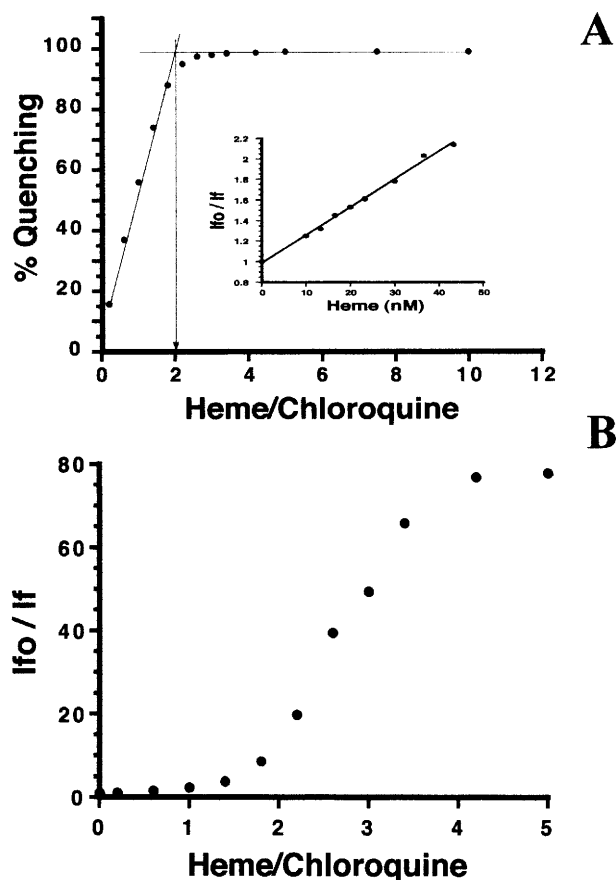
**Figure 2** Inhibition of haem–Pfhrp-2 complex formation by chloroquine

(A) Spectra of haem and Pfhrp-2 in the presence and absence of chloroquine. Haem alone (trace 1) has an absorption maximum at 385 nm that is shifted to 415 nm in the presence of Pfhrp-2 (trace 2). Addition of chloroquine to the haem–Pfhrp-2 complex (trace 3) results in the loss of the haem–Pfhrp-2 complex. When chloroquine is added before addition of haem (trace 4), a dramatic reduction in haem binding is observed and haem–chloroquine complex formation is indicated. When chloroquine is added to the haem–Pfhrp-2 complex a significant decrease in complex formation was observed, indicative of chloroquine extracting the haem bound to Pfhrp-2 to form a haem–chloroquine complex. Details of spectral recordings and reactions are described in the Experimental section. (B) Native PAGE analysis of the haem–Pfhrp-2 complex and its interaction with chloroquine. Coomassie Brilliant Blue staining (lanes 1–3) and DAB staining (specific for haem/haemoproteins; lanes 4–6). Lanes 1 and 4, Pfhrp-2; lanes 2 and 5, Pfhrp-2 + haem; lanes 3 and 6, Pfhrp-2 + chloroquine + haem. The diffuse band in lane 2 indicated haem–Pfhrp-2 complex formation, which was confirmed by specific haem peroxidase staining in lane 5. Addition of chloroquine resulted in the loss of haem–Pfhrp-2 complex formation as evidenced by normal movement on the Coomassie Brilliant Blue-stained gel (lane 3) and loss of haem peroxidase staining (lane 6). Details of the experiment are described in the Experimental section.

ally stains haem or haemoproteins. Pfhrp-2 alone did not stain with DAB (lane 4); however, in the sample containing haem and Pfhrp-2 a distinct staining for haem peroxidase activity was observed (lane 5), confirming the presence of haem–Pfhrp-2 complexes. Addition of chloroquine in this reaction resulted in almost total loss of peroxidase staining (lane 6), indicating removal of haem from the haem–Pfhrp-2 complex, and confirming the results from the Coomassie Brilliant Blue-stained gel (lane 3). The presence of Pfhrp-2 in all samples was confirmed by Western-blot analysis of these samples using anti-(Pfhrp-2) antibodies (results not shown). By using specific staining for haem we were able to visualize haem–Pfhrp-2 complexes on a gel and distinguish them from native Pfhrp-2 in our experiments, since Pfhrp-2 itself does not have any haem group and thus does not show peroxidase activity. Loss of peroxidase stain on treatment with chloroquine provided evidence for the removal of haem from haem–Pfhrp-2 complexes, indicating a possible mechanism of chloroquine action.

#### Haem–chloroquine binding

Haem–chloroquine binding was studied by monitoring the quenching of chloroquine fluorescence by haem. Data were plotted as the percentage quenching against the concentration



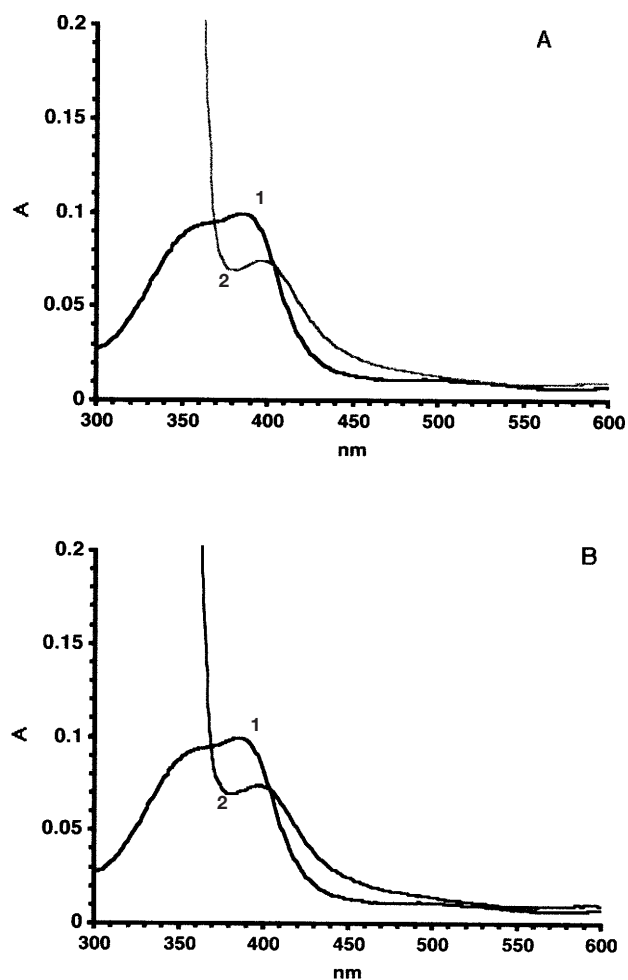
**Figure 3** Haem–chloroquine binding

(A) Direct plot of fractional quenching values against the haem/chloroquine ratio in solution. A typical curve for strong ligand binding is observed with a clear indication of saturation as a haem/chloroquine ratio of 2 is approached. (B) A Stern–Volmer plot of chloroquine fluorescence quenching by haem.  $I_0$  is the initial fluorescence intensity and  $I_1$  is the fluorescence intensity after addition of haem at a given point. Binding constants were calculated from linear regions of the curve. The inset in (A) shows the plot of  $I_0/I_1$  at low concentrations of chloroquine and haem. A  $K_d$  value of 37 nM was obtained for the haem–chloroquine complex from the slope of this plot. Deviation of the Stern–Volmer plot from linearity indicates the presence of more than one binding site, which was confirmed by results obtained from the direct plot shown in (A). For the titrations, 30  $\mu$ l of chloroquine from a stock of 100 nM was taken in a 3 ml cuvette and titrated with 1  $\mu$ l aliquots of 1  $\mu$ M haem solution (freshly prepared prior to use). Other details of the experiment are given in the Experimental section.

of haem in solution (Figure 3A). The results are typical of strong binding reactions with clear demarcation of the saturation point. A haem/chloroquine ratio of 2:1 was indicated. Data were analysed based upon two haem binding sites per molecule of chloroquine as described previously [29]. A Stern–Volmer plot of fluorescence intensities of the control and the haem-containing samples also showed deviation from a straight line indicating more than one haem binding site per chloroquine molecule (Figure 3B). The experiments were performed at very low concentrations of haem and chloroquine in order to calculate binding constants (Figure 3A inset). A  $K_d$  value of 37 nM was obtained for haem–chloroquine complex formation from the slope of this plot.

#### Interaction of chloroquine with Pfhrp-2

Equilibrium dialysis experiments failed to detect any significant interaction between chloroquine and Pfhrp-2. We dialysed 47  $\mu$ M



**Figure 4** Spectra of the haem–chloroquine complex and the effect of Pfhrp-2

(A) Spectra of haem before (trace 1) and after (trace 2) the addition of chloroquine (1 nmol of haem was mixed with 0.5 nmol of chloroquine). (B) Spectra of haem (trace 1) and haem–chloroquine (trace 2) in the presence of Pfhrp-2. Chloroquine was pre-incubated with Pfhrp-2 for 10 min before addition of haem.

Pfhrp-2 inside the dialysis tubing against 1 nM chloroquine outside. Even 100:1 binding would have resulted in an approx. 470-fold increase in chloroquine concentration inside the dialysis tubing containing Pfhrp-2. However, no significant increase in chloroquine concentration was detected indicating a lack of chloroquine binding by Pfhrp-2. In another experiment, where Pfhrp-2 preparations incubated with a 5 molar excess of chloroquine were dialysed against PBS and used for haem binding, no change in the amount of haem–Pfhrp-2 complex formation was detected (results not shown). This indicates that the haem binding sites of Pfhrp-2 are unaffected by chloroquine. Moreover, when haem–chloroquine binding was studied in the presence and absence of a 50-fold molar excess of Pfhrp-2, no change was observed in the haem–chloroquine interaction (Figure 4). These results indicate that haem has a higher affinity for chloroquine compared with Pfhrp-2. The observation is supported by a  $K_d$  value of  $3.7 \times 10^{-8}$  M for the haem–chloroquine complex as compared with a  $K_d$  value of  $9.4 \times 10^{-7}$  M for the haem–Pfhrp-2 complex.

**Table 1** Inhibition of malaria cysteine protease activity by haem, and its protection by Pfhrp-2

Malaria cysteine protease activity was measured by monitoring the release of fluorescent AMC from the specific peptide substrate Z-Phe-Arg-AMC. Concentrations of additives were as follows: haem, 50  $\mu\text{M}$ ; Pfhrp-2, 30  $\mu\text{M}$ ; chloroquine (CQ), 50  $\mu\text{M}$ ; E-64, 50  $\mu\text{M}$ . The assay was performed as described previously [25]. All additives were incubated with the enzyme for 30 min before addition of the substrate. E-64 is a highly potent and specific inhibitor of cysteine proteases. It was used as a positive control in the reaction.

Sample	$\Delta$ Fluorescence intensity (arbitrary units/min)
Control	12.5 $\pm$ 1.5
Control + haem	1.5 $\pm$ 0.7
Control + Pfhrp-2	12.1 $\pm$ 0.8
Control + Pfhrp-2 + haem	7.9 $\pm$ 0.8
Control + Pfhrp-2 + CQ + haem	1.4 $\pm$ 0.5
Control + CQ	11.7 $\pm$ 1.2
Control + CQ + haem	1.2 $\pm$ 0.5
Control + E-64	0.0

### Inhibition of cysteine protease by haem, and its antagonism by Pfhrp-2

Haem is a potent inhibitor of malaria cysteine protease activity [25]. Free haem released following haemoglobin digestion is detoxified by polymerization/degradation. The presence of haem-binding substances in parasite vacuoles would serve the purpose of haem detoxification and reduce the damage caused by haem. A drug that could interfere with haem detoxification will perturb this co-existence leading to haem toxicity. To test this hypothesis we assayed malaria cysteine protease (a crucial parasite enzyme known to be inhibited by haem) in the presence and absence of haem and chloroquine. A specific peptide substrate for malaria cysteine protease (Z-Phe-Arg-AMC) was used for the assay, and cleavage of fluorescent AMC from the peptide was monitored fluorimetrically. Haem inhibited the cysteine protease activity as shown by the reduction in the increase in fluorescence per minute. Inhibition of the malaria cysteine protease by haem in the presence of Pfhrp-2 was remarkably less than the control samples without Pfhrp-2 (Table 1). The presence of chloroquine in the reaction mixture reversed this effect and the inhibition returned to its normal level, indicating disruption of the haem detoxification function of Pfhrp-2. The presence of both haem and chloroquine without Pfhrp-2 caused somewhat higher inhibition. Under normal circumstances, toxicity of haem would be countered by haem-binding proteins, however, in the presence of a drug such as chloroquine that has a higher affinity for haem, this protection would be lost and lead to the disruption of parasite metabolism by haem/haem–drug complexes.

### Conclusions

In the present study we have examined the binding of haem to Pfhrp-2, the major haem-binding protein present in the malaria parasite. For proteins with very high affinities for haem, such as haemopexin and haemoglobin, only approximate estimates of  $K_d$  values are reported, which mostly fall in the pM range. The  $K_d$  value calculated for the haem–Pfhrp-2 complex is 0.94  $\mu\text{M}$ . The strong haem-binding ability of this protein could provide the malaria parasite with an important source with which to combat haem toxicity, by storage of toxic haem until it can be processed by other means [30,31].

Malaria pigment has long been associated with the action of chloroquine. The first morphological effect of chloroquine treatment on the malaria parasite is the clumping of haemozoin pigment [32,33]. Our results described in the present study suggest that chloroquine interacts with haem bound to Pfhrp-2 and during this process a haem–chloroquine complex is formed. Since very little haem is sufficient to disrupt the biochemical processes of the parasite, even a small shift in the haem detoxification process towards free haem would be enough to cause damage to the parasite [34].

Moreover, haem bound to Pfhrp-2 may lose its toxic effects on parasite enzymes as observed by us in cysteine protease experiments. A protein such as Pfhrp-2 that has a large number of haem binding sites would serve as an ideal sink for the free haem and protect the parasite from its toxicity. It is probable that the malarial parasite might be using such proteins as a first line of defence against haem toxicity. Our results showing antagonism of haem damage to the cysteine protease by Pfhrp-2 and the loss of this protection in the presence of chloroquine seem to support this hypothesis.

The observations that chloroquine can extract haem bound to Pfhrp-2, the major protein involved in haem detoxification, have provided an insight into the molecular interactions involved in the mechanism of action of this drug. Inhibition of haem–Pfhrp-2 complex formation could be used for the screening of potential inhibitors of the haem detoxification system of the malaria parasite.

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