

Intraerythrocytic *Plasmodium falciparum* Expresses a High Affinity Facilitative Hexose Transporter*

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Charles J. Woodrow‡, Jeffrey I. Penny, and Sanjeev Krishna§

From the Department of Infectious Diseases, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, United Kingdom

Asexual stages of *Plasmodium falciparum* cause severe malaria and are dependent upon host glucose for energy. We have identified a glucose transporter of *P. falciparum* (PfHT1) and studied its function and expression during parasite development *in vitro*. PfHT1 is a saturable, sodium-independent, and stereospecific transporter, which is inhibited by cytochalasin B, and has a relatively high affinity for glucose ($K_m = 0.48$ mM) when expressed in *Xenopus laevis* oocytes. Competition experiments with glucose analogues show that hydroxyl groups at positions C-3 and C-4 are important for ligand binding. mRNA levels for PfHT1, assessed by the quantitative technique of tandem competitive polymerase chain reaction, are highest during the small ring stages of infection and lowest in gametocytes. Confocal immunofluorescence microscopy localizes PfHT1 to the region of the parasite plasma membrane and not to host structures. These findings have implications for development of new drug targets in malaria as well as for understanding of the pathophysiology of severe infection. When hypoglycemia complicates malaria, modeling studies suggest that the high affinity of PfHT1 is likely to increase the relative proportion of glucose taken up by parasites and thereby worsen the clinical condition.

Malaria is one of the most important pathogenic protozoa and is responsible for more than 1 million deaths each year. Antimalarial drug resistance is developing rapidly in different geographical areas and is severely curtailing therapeutic options (1). Asexual multiplication of intraerythrocytic *Plasmodium falciparum* is a prerequisite for the development of clinical symptoms and lethal outcome in malarial infection (2). At this stage, parasites are dependent on glucose from the host as a source of energy (3) and metabolize glucose anaerobically. Increases in metabolic demands are associated with enlargement of parasites within erythrocytes before the stage of nuclear division (4). There is an approximately 100-fold increase in the utilization of glucose by maturing parasites when compared with uninfected erythrocytes. This induced increase in uptake of glucose is accompanied by increased production and export of lactic acid by infected cells (4).

Studies on intact infected erythrocytes have suggested that *P. falciparum* obtains its glucose through an equilibrative

mechanism (5) which may involve a saturable carrier associated with the parasite itself (6). However, these types of studies assess mechanisms of glucose transport indirectly, because multiple membrane systems are involved in analysis. More detailed assessment of the enzymatic characteristics of a proposed glucose transporter from the malarial parasite can only be individually assessed in heterologous expression systems.

Previously, we have hypothesized that asexual stages of parasites encode substrate-specific transporters that are located in the region of the developing parasite's plasma membrane (7). These transporters are presumed to act in conjunction with important changes in the permeability properties of the infected erythrocyte membrane to regulate the uptake of substrates by parasites. As a preliminary test of this hypothesis, we microinjected *Xenopus laevis* oocytes with mRNA obtained from cultured *P. falciparum* and demonstrated significantly increased uptake of several substrates or analogues of metabolism including 2'-deoxy-D-glucose (2-DOG)¹ and lactate (7).

We have now identified a parasite-encoded hexose transporter that is localized to the region of the parasite plasma membrane within the infected red cell. Quantitation of mRNA encoding this transporter during the asexual and gametocyte stages of the parasite's life cycle suggests that its expression is under developmental control. Functional studies on this transporter in *Xenopus* oocytes have confirmed that it is a facilitative transporter with relatively high affinity for glucose. These findings have identified a key parasite-encoded substrate transporter that is a potentially novel drug target, as well as establishing the value of a heterologous expression system for the study of malarial transport proteins.

EXPERIMENTAL PROCEDURES

Identification and Cloning of PfHT1 Sequence—Sequence data were obtained through early release from The Institute for Genomic Research at www.tigr.org and/or NCBI at www.ncbi.nlm.nih.gov by searching using TBLASTN and published sequences for GLUT1 (human facilitative glucose transporter) (8) and SGLT1 (rat sodium-dependent glucose transporter) (9). PCR on genomic DNA from parasite clone 3D7 using *Pfu* polymerase (Stratagene, La Jolla, CA) was carried out with primers designed to introduce *Bgl*II restriction sites and a strong eukaryotic Kozak consensus (AATAATG to CACCATG, where the initiation codon is underlined). The product was subcloned into *Bgl*II sites in pSPGT1, which contains 5'- and 3'-untranslated *Xenopus* β -globin sequences (10). The final product was verified by sequence analysis.

Expression of PfHT1 in *Xenopus* Oocytes, Kinetic Analyses, and Studies with Competitors and Inhibitors—*X. laevis* oocytes were prepared and used in uptake assays as described previously (7). cRNA encoding

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AJ131457.

‡ Wellcome Trust Clinical Training Fellow.

§ Wellcome Trust Senior Research Fellow in Clinical Science. To whom correspondence should be addressed. Tel.: 44-181-725-5836; Fax: 44-181-725-3487; E-mail: s.krishna@sghms.ac.uk.

¹ The abbreviations used are: 2-DOG, 2'-deoxy-D-glucose; PfHT1, *Plasmodium falciparum* hexose transporter 1; GLUT1, human facilitative glucose transporter 1; 6-DOG, 6'-deoxy-D-glucose; PCR, polymerase chain reaction; TC-PCR, tandem competitive PCR; K_m , half-maximal inhibition constant for carrier transport; PBS, phosphate-buffered saline.

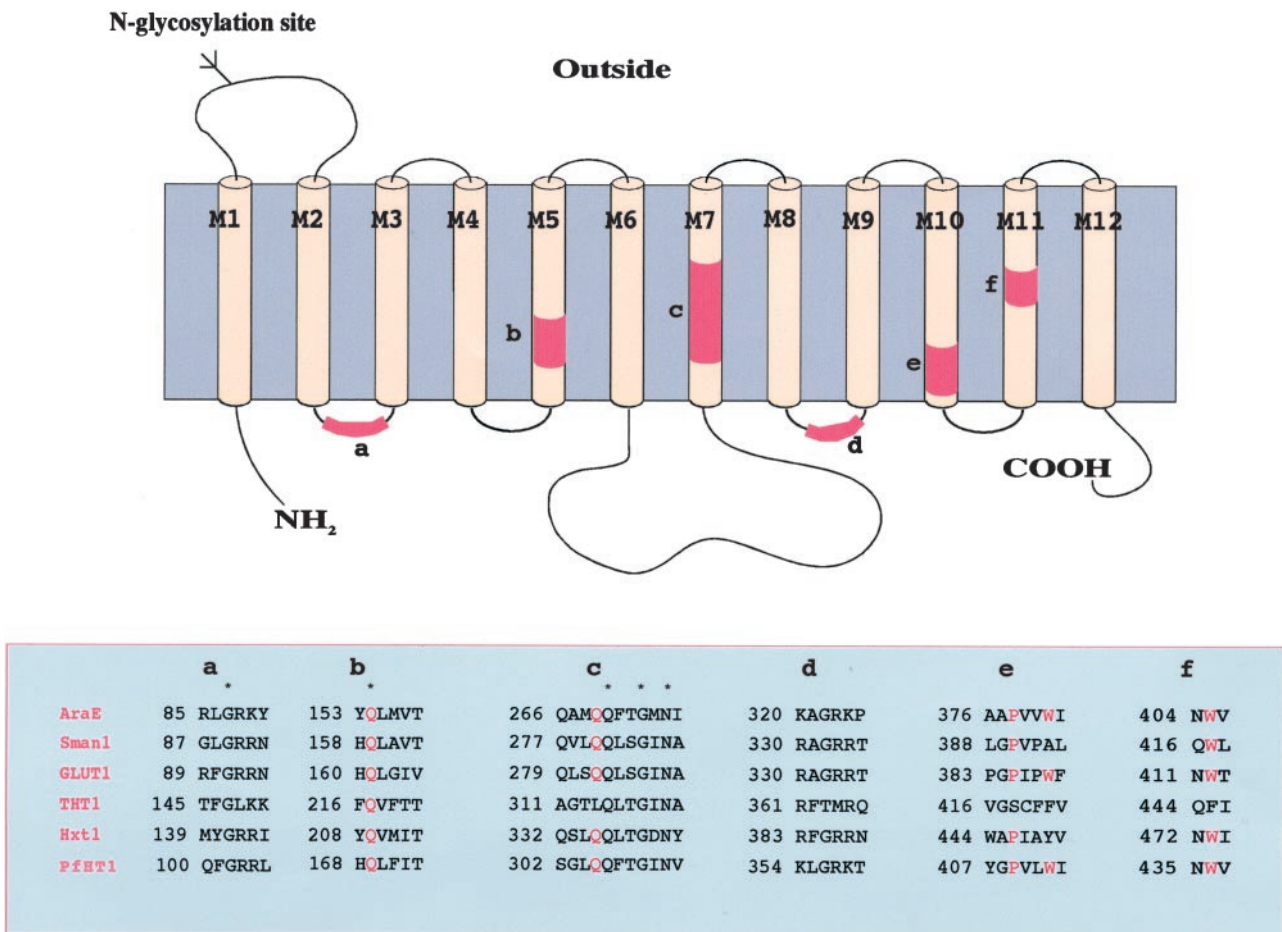


FIG. 1. **Sequence analysis of PfHT1.** Top panel, schematic model for the membrane arrangement of PfHT1 based on Mueckler's original proposal (8). Numbering (M1–M12) refers to transmembrane helices. A potential N-glycosylation site is indicated (69NCS). Regions (a–f) highlighted in red are shown in greater detail in the bottom panel. Bottom panel, examples of aligned sequences conserved between PfHT1 and sequences from the sugar transporter superfamily from phylogenetically distant organisms. Accession numbers in GenBank™ for each sequence with the percentage of identical residues compared with PfHT1 are as follows: AraE, *Escherichia coli* arabinose transporter (P09830; 27.2%); Sman1, *Schistosoma mansoni* glucose transporter protein 1 (L25065; 24.5%); GLUT1, human glucose transporter 1 (K03195; 29.8%); THT1, *Trypanosoma brucei* hexose transporter 1 (M81386; 20.0%); Hxt1, *Saccharomyces cerevisiae* glucose transporter 1 (P32465; 26.6%); PfHT1, *P. falciparum* hexose transporter. Asterisks indicate residues that are conserved throughout these sequences. Residues highlighted in red are of functional significance as determined by mutagenesis experiments (23–26). Regions a and d contain a GRR/K motif in positions characteristic of this superfamily of transporters. Regions b and c contain highly conserved glutamine residues that participate in exofacial ligand binding. Region e contains a proline residue that is required for conformational flexibility, and a tryptophan residue that is involved in binding to cytochalasin B. Region f contains a tryptophan residue essential for transport activity (26).

PfHT1 transcribed (MEGAscript™ SP6, Ambion, Austin, TX) from *Xba*I-linearized template (10 ng in 25 nl of water) or RNase-free water was injected into oocytes, and uptake studies were carried out 18–48 h later. For kinetic studies, uptakes of 2'-deoxy-[6-¹⁴C]D-glucose (2-DOG) (58 mCi·mmol⁻¹) and [U-¹⁴C]D-glucose (310 mCi·mmol⁻¹) (Amersham Pharmacia Biotech, Amersham, UK) were measured after 5 min and corrected for uptake into water-injected controls. Uptakes are linear during this interval (data not shown). Kinetic parameters were estimated by using a Michaelis-Menten model (PRISM™ v.2, GraphPad Software Inc., San Diego, CA). For studies with competitors and inhibitors (all from Sigma), uptakes were carried out for 30 min with radiolabeled permeants (14.3 μM 2-DOG, or 2.69 μM D-glucose) and 35 μM unlabeled D-glucose.

To determine the *K_i* (half-maximal inhibition constant for carrier transport) for 6'-deoxy-D-glucose (6-DOG), radiolabeled D-glucose was at a concentration of 3 μM and uptakes were for 10 min with a range of 6-DOG concentrations. Data were analyzed using a one-site competition model and were corrected for uptake into water-injected controls.

mRNA and Southern Blot Analyses—Radioactive probes for Southern and Northern blot analyses used full-length *PfHT1* sequence. To construct a competitor plasmid for quantitative PCR (tandem competitive PCR (TC-PCR) (11), we used oligonucleotide restriction-site mutagenesis to generate a 515-base pair fragment of *PfHT1* containing a novel, asymmetrical *Eco*RI site. This product, corresponding to nucleotide positions 1001–1515 of the open reading frame of *PfHT1*, was

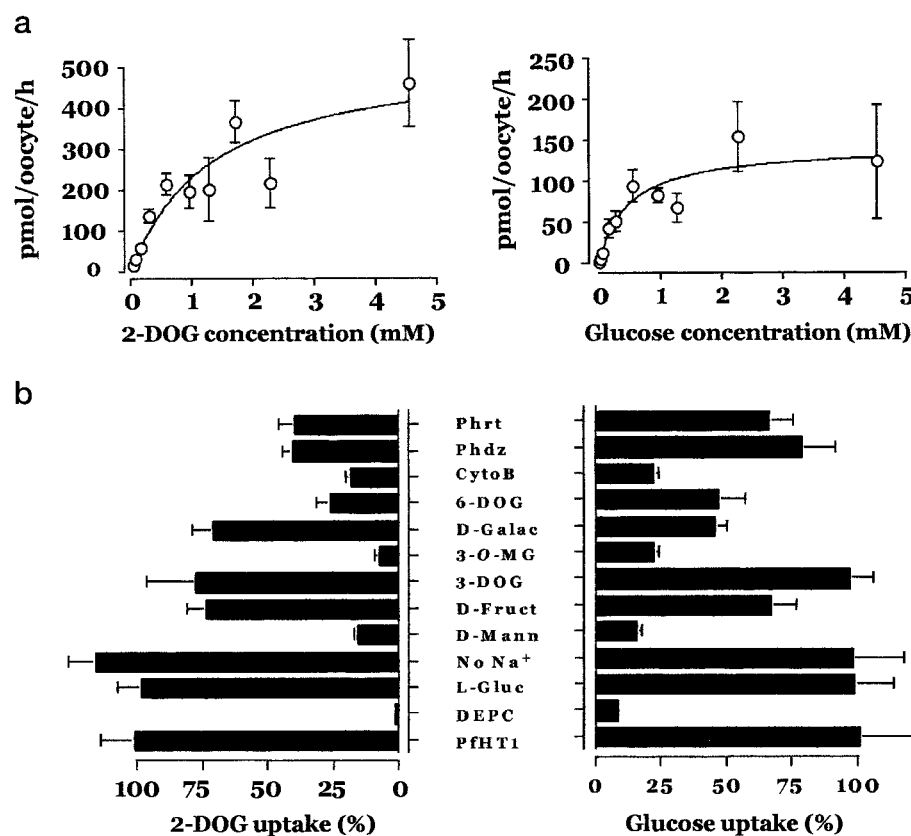
ligated into a construct containing a similarly mutated β-tubulin sequence (a "housekeeping" gene) (12).

Cultures of *P. falciparum* (clone 3D7) were synchronized to an 8-h time window by sequential Percoll centrifugation and sorbitol lysis (13). Samples for RNA analysis were collected every 8 h into RNA Isolator™ (GenoSys Biotechnologies, Cambridge, UK). Gametocyte-enriched culture was harvested in a similar fashion. Contamination of parasite cultures with *Mycoplasma* spp. was excluded by PCR (Stratagene).

To quantify the amount of cDNA for PfHT1 (or for β-tubulin), cDNA was mixed with a series of dilutions of competitor plasmid, and after PCR the product was digested with *Eco*RI and quantified on a GDS 7600 system (Ultra-Violet Products, Upland, CA). After correction for heteroduplex formation (12), the plasmid competitor/cDNA ratio was calculated for individual reactions and analyzed as exemplified in Fig. 5. To exclude the possibility of DNA contaminating mRNA preparations, PCR was carried out across an intron in β-tubulin sequence.

Generation of Anti-peptide Antibodies and Immunolocalization—Peptide sequence KDICSNEGKKGKSG (Hpep1, corresponding to residues 6–21 in PfHT1, GenoSys Biotechnologies) was selected for immunolocalization studies, because it lies in the amino-terminal non-membranous segment of PfHT1 and shows no homology with any other glucose transporter or malaria protein. After conjugation to keyhole limpet hemocyanin, rabbit polyclonal antibodies were generated by immunizing rabbits with peptide (150 μg) in Freund's complete adjuvant, followed by four equivalent doses administered in Freund's in-

FIG. 2. Expression of PfHT1 in *Xenopus laevis* oocytes. Panel a, kinetic analyses. Initial mean (8 oocytes per concentration, mean \pm S.E.) uptake rates of 2-DOG (left panel) and D-glucose (right panel) are plotted against concentration of substrate. Panel b, studies with competitors and inhibitors. Uptakes (8 oocytes per condition) were carried out with radiolabeled permeants 2-DOG (left panel) or D-glucose (right panel). Results are expressed as mean \pm S.E. percentage of uptake of substrate for each condition compared with uptake in uncompeted oocytes. PfHT1, malarial hexose transporter without competitor or inhibitor; DEPC, RNase-free water-injected control oocytes; L-Gluc, L-glucose (10 mM); No Na⁺, sodium-free Barth's medium where Na⁺ is replaced with equimolar choline chloride; D-Mann, D-mannose (10 mM); D-Fruct, D-fructose (10 mM); 3-DOG, 3'-deoxy-D-glucose (10 mM); 3-O-MG, 3'-O-methyl-D-glucose (10 mM); D-Galac, D-galactose (10 mM); 6-DOG, 6'-deoxy-D-glucose (10 mM); CytoB, cytochalasin B (50 μ M); Phdz, phloridzin (500 μ M); Phrt, phloretin (150 μ M).



complete adjuvant at 2-week intervals and a final dose of peptide alone.

P. falciparum cultures (10% infected red cells) were washed in serum-free RPMI 1640 and fixed onto slides in methanol at -70°C . Additionally, aliquots were applied to slides coated with poly-L-lysine and permeabilized with acetone (14). Antiserum (20 μ l) was incubated (1 h at 37°C) with an equivalent volume of PBS, PBS containing cognate peptide (50 mg/ml), or an irrelevant peptide (50 mg/ml) before application to slides. After washing (once in PBS and three times in PBS with fetal calf serum, 1% (v/v)), secondary fluorescein isothiocyanate-conjugated swine anti-rabbit antibody (10 μ l) was applied for 1 h and washing was repeated. Fluorescence was visualized on a Zeiss Axiovert 100 confocal microscope.

For Western blotting, parasites were washed in RPMI 1640 medium and lysed in hypotonic medium (10 mM $\text{Na}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$, pH 7.4) containing proteinase inhibitors (phenylmethylsulfonyl fluoride (1 mM), *N* α -*p*-tosyl-L-lysine chloromethyl ketone (0.2 mM), *N*-tosyl-L-phenylalanine chloromethyl ketone (0.1 mM), antipain (0.1 mg/ml), leupeptin (0.01 mg/ml); all from Sigma). After extraction with Triton X-100, samples were resolved by 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose, incubated with primary antibody, and washed, and the blot developed according to manufacturer's instructions (ECL, Amersham). Blocking was with fat-free milk (5% in PBS).

RESULTS

Sequence Analysis of PfHT1—A continuous open reading frame with significant homology to GLUT1, a human facilitative glucose transporter, was identified on contig 7290 of chromosome 2 of *P. falciparum* (see "Experimental Procedures") and designated *PfHT1*. Hybridization of genomic *P. falciparum* Southern blots, using *PfHT1* sequence as probe at low stringency, did not reveal any other closely related sequences (data not shown). *PfHT1* predicts a 504-amino acid polypeptide (56.4 kDa) with 12 membrane-spanning helices and many features, including its length, which are in common with members of the facilitative glucose transporter superfamily. There is 20–29.8% identity (51.5–58.5% similarity) between PfHT1 and other representatives from this family, which include examples from both prokaryotes and eukaryotes (Fig. 1). PfHT1 contains several residues and motifs essential for exofacial and endofacial

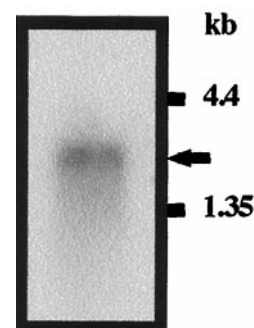


FIG. 3. Northern blot of *PfHT1*. mRNA (4 μ g) obtained from asynchronous parasites (clone 3D7) was hybridized to *PfHT1*, washed at low stringency ($2\times$ SSC, 0.1% SDS, at 40°C for 30 min), and visualized on a PhosphorImager after overnight exposure.

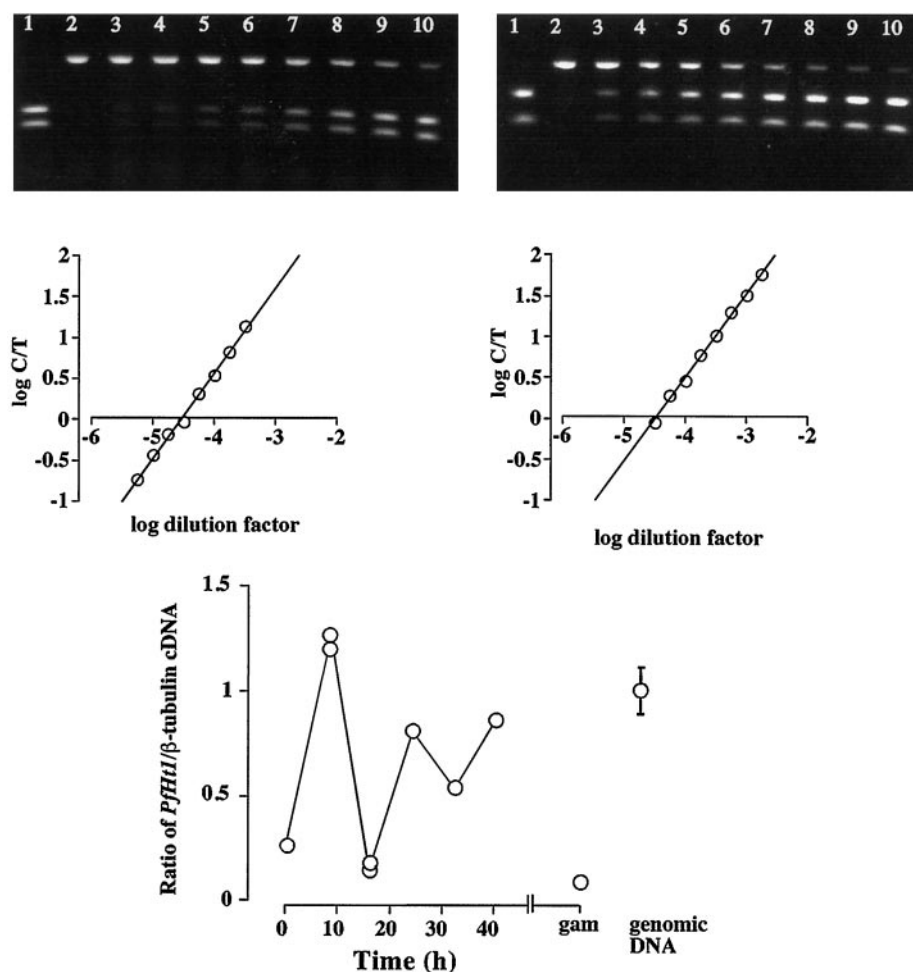
ligand binding and substrate transport, some of which are shown in Fig. 1. There is one potential N-glycosylation site in the first extracellular loop of PfHT1. PfHT1 does not contain any tandemly repeated motifs, which are characteristic of many malarial proteins.

Functional Characterization of PfHT1—To establish and characterize the function and biochemical properties of PfHT1 we introduced a strong eukaryotic Kozak consensus sequence at the start of *PfHT1* and cloned it into a vector used for expression studies in *Xenopus* oocytes (see "Experimental Procedures"). cRNA transcribed *in vitro* and microinjected into oocytes induced large increases in uptake of D-glucose and 2-DOG. For example, in one experiment the mean uptake of 2-DOG in PfHT1-injected oocytes was 19.2 pmol/oocyte/h and in water-injected controls it was 0.13 pmol/oocyte/h, an ~ 150 -fold increase in uptake.

To confirm that PfHT1 belongs to the family of hexose transporters, we examined its enzymatic characteristics. The uptake

FIG. 4. **Quantitative mRNA studies.**

Top left panel, example of results of TC-PCR for PfHT1 with track 1, competitor alone; track 2, cDNA alone; and tracks 3–10, increasing one-fourth log concentrations of competitor plasmid with constant cDNA concentrations (5 ng) in each PCR. Products derived from cDNA remain undigested by *EcoRI*, whereas those derived from competitor are cleaved. *Top right panel*, same as for *top left panel*, but with β -tubulin. *Middle panel*, quantitation of bands from respective *top panels* (C, competitor-derived product; T, cDNA-derived product). C/T ratios are analyzed by linear regression, where the X-intercept represents equivalence in concentrations of cDNA and competitor (here, 4.55 and 4.49 for PfHT1 and β -tubulin, respectively, giving a ratio of PfHT1/ β -tubulin = 0.83, T = 40 h, see below). *Bottom panel*, the ratio of PfHT1 to β -tubulin cDNA in samples obtained from synchronized parasite cultures following invasion (0 h). Samples were collected at eight hourly intervals. The ratios of mRNAs at the 8- and 16-h time points were estimated in two independent experiments. Gam, gametocyte-rich preparations (70% gametocytes).



of permeants (2-DOG and glucose) was saturable at relatively low concentrations of glucose (<5 mM) and permitted detailed kinetic analyses. For 2-DOG, $K_m = 1.31$ mM and $V_{max} = 535$ pmol/oocyte/h and for D-glucose $K_m = 0.48$ mM and $V_{max} = 143$ pmol/oocyte/h (Fig. 2a). PfHT1 has a much higher affinity for both these permeants when compared with GLUT1, the principle erythrocyte transporter, which has a K_m (2-DOG) = 6.9 mM (15) (also expressed in oocytes).

PfHT1 is stereospecific for D-glucose, because an excess of L-glucose in the medium used for uptake assays did not interfere with uptake of either 2-DOG or D-glucose (Fig. 2b). There is no requirement for extracellular sodium, because substitution of Na^+ ions with choline did not interfere with the function of PfHT1. These findings are consistent with the primary sequence of PfHT1, which predicts it to be a facilitative hexose transporter.

Structural Requirements in Substrates for Transport by PfHT1—We studied the structural requirements for ligand interactions between PfHT1 and hexoses by using a variety of competitors and inhibitors. The hydroxyl groups at C-3 and C-4 positions in glucose are important in uptake by PfHT1 as an excess of 3'-deoxy-D-glucose or D-galactose (the 4-epimer of glucose; both at 10 mM) competed relatively poorly when glucose or 2-DOG were used as permeants (Fig. 2b). In particular, the oxygen atom at the C-3 hydroxyl moiety is likely to be a hydrogen bond recipient, because 3-O-methylglucose restored the capacity to inhibit transport of both permeants. In contrast, D-mannose (the 2-epimer of glucose; 10 mM) competed well with both 2-DOG and D-glucose for PfHT1 uptake into oocytes. The mean (\pm S.E.) K_i for 6-DOG (using D-glucose as permeant) in three independent experiments was 2.2 ± 0.86 mM (data not

shown). The relatively small effects of changes in the C-2 and C-6 positions in these experiments suggest that these positions contribute little to PfHT1 functionality. Fructose consistently inhibited uptake of D-glucose and 2-DOG by 25–35%, suggesting that in contrast to GLUT1, PfHT1 may transport fructose. PfHT1 is also inhibited by cytochalasin B (50 μ M) and contains a tryptophan residue implicated in binding to this inhibitor (Fig. 1).

Stage-specific Expression of PfHT1 mRNA—mRNA obtained from asynchronous *P. falciparum* contains a single species (~2.6 kilobases) when hybridized to PfHT1 at low stringency (Fig. 3). We characterized the pattern of PfHT1 mRNA expression in greater detail using synchronized cultures of asexual stages, as well as gametocytes. The technique of TC-PCR was used to quantitate precisely the amount of PfHT1 mRNA relative to that of a housekeeping gene, β -tubulin (12) (Fig. 4). The precision and reproducibility of this ratiometric method were confirmed by assessing the gene copy number of PfHT1 relative to that for β -tubulin using genomic DNA as template in five independent TC-PCR experiments (mean \pm S.E. ratio = 1.07 ± 0.12 , Fig. 4, lower panel).

The expression of PfHT1 is clearly under strict developmental control during the life cycle of *P. falciparum* with an early peak of mRNA expression at the small ring stage (8 h time point, Fig. 4, lower panel). The 8-h time point represents parasites developing 8–16 h after invasion of erythrocytes, with the majority developing at ~12 h.

At 16 h after invasion, there is a fall by ~1 \log_{10} unit in the ratio of PfHT1/ β -tubulin mRNA. Levels of mRNA for PfHT1 again increase relative to β -tubulin when parasites develop

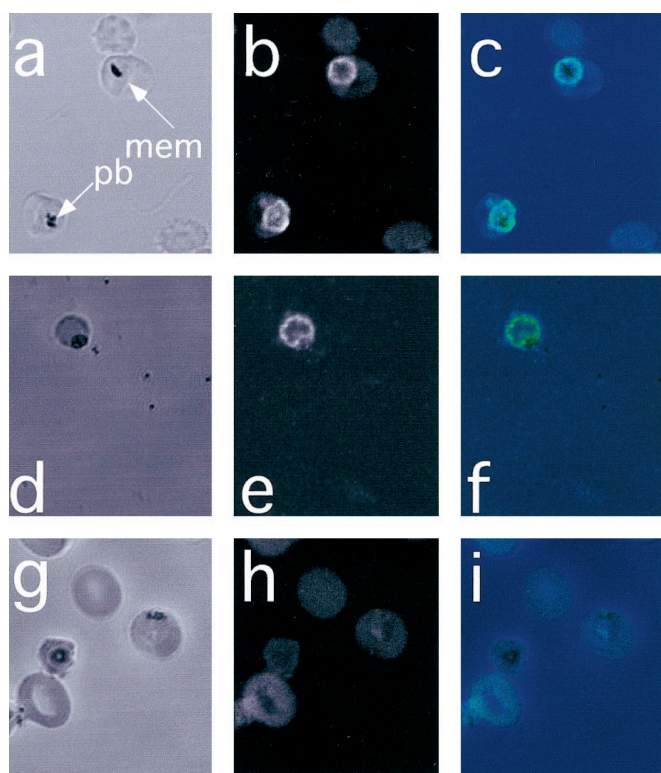


FIG. 5. **Immunolocalization of PfHT1 with anti-Hpep1.** Panels *a-c*, bright-field, immunofluorescence, and combined images of intraerythrocytic *P. falciparum* (mature trophozoites) stained with anti-Hpep1 antibody. pb, pigment body; mem, parasite-host interface. Panels *d-f*, images corresponding to panels *a-c* of free parasites (mature trophozoite). Panels *g-i*, images corresponding to panels *a-c* with anti-Hpep1 antibody pre-incubated with excess of cognate peptide.

into more mature stages. Trophozoites and meronts express intermediate levels of mRNA for *PfHT1* compared with the preceding peak and trough levels. Gametocytes express the lowest ratio of *PfHT1*/β-tubulin. To confirm the early peak in *PfHT1* expression, TC-PCR was repeated for the 8- and 16-h time points and each ratio was closely reproduced, further demonstrating the precision of this assay (Fig. 4, lower panel).

Immunolocalization of PfHT1—There was no staining of the infected erythrocyte membrane or red cell cytosol (Fig. 5, *a-c*) when polyclonal antibodies raised to a Hpep1 were applied to infected erythrocytes. Staining of the parasite within the erythrocyte outlined the region of the parasite plasma membrane and was most intense when parasites were free of surrounding structures (Fig. 5, *d-f*). In some preparations, staining was also associated with the pigment body. Staining was abolished by pre-incubation of antiserum with excess cognate peptide (Hpep1, Fig. 5, *g-i*) but not after incubation with irrelevant peptide (not shown).

PfHT1 was also recognized on Western blots made from parasites lysed with hypotonic buffer in Triton-soluble (Fig. 6), and to a lesser extent, in Triton-insoluble fractions. Recognition was abolished by pre-incubation of antiserum with excess Hpep1 (not shown). The estimated molecular mass of PfHT1 (62 kDa), which contains a potential *N*-glycosylation site, is consistent with its predicted mass (56.4 kDa).

DISCUSSION

Glucose is the main energy source for asexual stages of *P. falciparum* (3). The isolation of a novel parasite-encoded hexose transporter is therefore of fundamental biological interest. A number of findings suggest that PfHT1 is the major hexose transporter for intraerythrocytic parasites. We have estab-

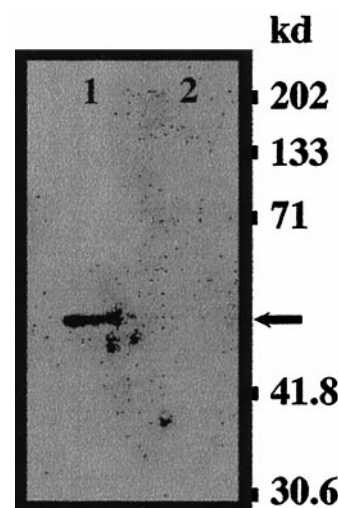


FIG. 6. **Western blot of PfHT1.** Track 1, Triton-soluble fraction (4 μg) of infected erythrocytes (ring stages, 62%; trophozoites, 35%; meronts, 3%) lysed in hypotonic buffer and incubated with anti-PfHT1 antibody after transfer to membrane. Track 2, equivalent amount of material from uninfected erythrocytes treated in the same way as for track 1.

lished that *PfHT1* is a single copy gene with no close homologues of *PfHT1* discernible from examination of both Southern and Northern blots under conditions of low stringency for hybridization and washing. PfHT1 is expressed as a single transcript (Fig. 3) that varies considerably in abundance during the life cycle of *P. falciparum*. The pattern of expression of *PfHT1* mRNA, which peaks sharply 8 h after invasion of the red cell (Fig. 4), is consistent with anticipation of a rapid rise in glucose consumption as ring forms mature to become trophozoites (4). A sustained secondary rise in *PfHT1* mRNA levels may serve to maintain glucose supply as parasites begin to divide. Thus the variation in abundance of mRNA encoding PfHT1 is consistent with the changes in glucose utilization as asexual stage parasites mature in cells. Interestingly, gametocytes expressed the lowest relative abundance of *PfHT1* mRNA suggesting that high levels of glucose uptake may not be critical at this stage of the infection.

When expressed in *Xenopus* oocytes, PfHT1 mediates sodium-independent uptake of hexoses (Fig. 2). The uptake of glucose analogues in intact *P. falciparum*-infected erythrocytes is also sodium-independent (5) and saturable (6). Furthermore, the predicted K_m for 6-DOG uptake into parasites (~5 mM) derived after modeling from studies in infected erythrocytes (6) is consistent with our K_i estimate for 6-DOG (2.2 mM). Taken together these observations support assignment of PfHT1 as the principal hexose transporter in asexual stages of parasite development. A number of controversial mechanisms have been proposed to explain the increase in permeability of host-derived structures to metabolites of parasites (16–19). Whatever the nature of these important mechanisms, primary regulation of nutrient uptake and export of products of metabolism takes place across the parasite plasma membrane, because axenically grown parasites are capable of completing the asexual stage of development (20). Localization by confocal microscopy and Western blotting experiments demonstrate that PfHT1 is associated with the parasite plasma membrane and not the erythrocyte membrane. PfHT1 therefore provides an example of a molecular mechanism for transport of substrates into the parasite, in which substrate-specific transporters act in conjunction with alterations in surrounding membrane structures to supply nutrients to the parasite (7).

Heterologous expression systems avoid the difficulties of

studying transport across the multiple membranes of the infected erythrocyte and will become increasingly important as the malaria-sequencing initiative evolves. The high AT content of parasite DNA does not interfere with expression in the *Xenopus* oocyte system.

The isolation of a parasite-encoded facilitative hexose transporter permits studies focussing on differences between the well characterized human hexose transporter family of sequences (GLUT1 to GLUT5) and the newly isolated malarial homologue. We have already shown that PfHT1 requires an appropriately positioned hydroxyl group in the C-4 position of hexoses, a property which distinguishes it from human GLUT1 (21). Such differences may be used as a basis for the design of compounds selectively toxic to the parasite.

The >10-fold higher affinity for D-glucose shown by PfHT1 compared with GLUT1 may result in important physiological consequences. Infected erythrocytes adhere to microvasculature when parasites mature giving rise to organ-specific complications such as cerebral malaria and the metabolic complications of hypoglycemia and lactic acidosis (2). The enzymatic properties of PfHT1 suggest that there is potential for infected erythrocytes to divert this substrate away from host tissues. This may be particularly important in cerebral microvessels, because the brain relies on glucose, which is transported by GLUT1 through the blood-brain barrier (22). The magnitude of this metabolic diversion is further predicted to increase during episodes of hypoglycemia (plasma glucose ≤ 2.2 mM). These observations may also explain how parasites continue to develop even when glucose delivery becomes rate-limiting for cerebral metabolism, for example during episodes of hypoglycemia which frequently (~20% of cases) (2) complicate the condition of cerebral malaria.

Our characterization of a *P. falciparum*-encoded high affinity hexose transporter identifies an attractive potential drug target because antimalarials used in the management of severe malaria take many hours to achieve maximum inhibition of glucose utilization (13). Inhibitors of PfHT1 may be rapidly parasitocidal as well as interfering with pathophysiological processes in cerebral malaria.

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