Comparative characterization of hexose transporters of *Plasmodium knowlesi*, *Plasmodium yoelii* and *Toxoplasma gondii* highlights functional differences within the apicomplexan family

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Chemotherapy of apicomplexan parasites is limited by emerging drug resistance or lack of novel targets. PfHT1, the *Plasmodium falciparum* hexose transporter 1, is a promising new drug target because asexual-stage malarial parasites depend wholly on glucose for energy. We have performed a comparative functional characterization of PfHT1 and hexose transporters of the simian malarial parasite *P. knowlesi* (PkHT1), the rodent parasite *P. yoelii* (PyHT1) and the human apicomplexan parasite *Toxoplasma gondii* (*T. gondii* glucose transporter 1, TgGT1). PkHT1 and PyHT1 share $> 70\,\%$ amino acid identity with PfHT1, while TgGT1 is more divergent ($37.2\,\%$ identity). All transporters mediate uptake of D-glucose and D-fructose. PyHT1 has an affinity for glucose ($K_m \approx 0.12$ mM) that is higher than that for PkHT1 ($K_m \approx 0.67$ mM) or PfHT1 ($K_m \approx 1$ mM). TgGT1 is highly temperature dependent (the Q_{10} value, the fold change in activity for a 10 °C change in temperature, was > 7) compared with *Plasmodium* transporters (Q_{10}, 1.5–2.5), and overall has the highest affinity for glucose ($K_m \approx 30\ \mu$M). Using active analogues in competition for glucose uptake, experiments show that hydroxyl groups at the C-3, C-4 and C-6 positions are important in interacting with PkHT1, PyHT1 and TgGT1. This study defines models useful to study the biology of apicomplexan hexose permeation pathways, as well as contributing to drug development.

Key words: glucose, malaria, oocyte, transport, *Xenopus*.

INTRODUCTION

Apicomplexan organisms are intracellular protozoan pathogens responsible for medical and veterinary diseases of huge global significance. *Plasmodium falciparum* is the most important cause of malaria, which kills 1 million children each year. Together with three other *Plasmodium* spp., malaria also afflicts hundreds of millions of individuals, causing serious morbidity [1]. *Toxoplasma gondii* is an opportunistic pathogen that has become particularly important in the era of AIDS. Toxoplasmosis is a frequent cause of cerebral pathology in immunocompromised individuals, and can produce severe congenital anomalies. Other apicomplexan organisms such as *Cryptosporidium parvum* and *Cyclospora* spp. also cause opportunistic infections in humans, whereas animals are commonly infected by *Theileria*, *Sarcocystis* and *Babesia* spp. [2]. Drugs to treat these organisms are limited, either by emerging resistance or because adequate targets have not yet been identified and exploited [3].

We have focused on hexose transport as a potential drug target for apicomplexan organisms for the following reasons. Both *Plasmodium* and *Toxoplasma* are wholly dependent on hexoses for energy during their pathogenic stages of infection (respectively the asexual intra-erythrocytic and tachyzoite stages) [4,5]. They utilize glucose predominantly by the relatively inefficient process of anaerobic glycolysis. In *Plasmodium*-infected erythrocytes glucose consumption is greatly increased compared with uninfected red cells, and a continuous supply of glucose is essential for survival of parasites, which do not contain energy stores. Consequently, intraparasitic ATP concentrations and pH fall within minutes of cultured parasites being deprived of glucose [4].

To exploit hexose transporter 1 encoded by *P. falciparum* (PfHT1) as a potential drug target we previously cloned this single-copy gene and showed that it mediates uptake of physiologically relevant hexoses (including fructose), by using the *Xenopus laevis* oocyte heterologous expression system [6]. Our findings have translated back to experiments on parasite cultures where fructose can replace glucose as an energy source for parasites [7].

To increase understanding of hexose transport in apicomplexan organisms, we have now carried out a comparative analysis of hexose transporters encoded by *T. gondii* [*T. gondii* glucose transporter 1 (TgGT1)], the simian malaria parasite *P. knowlesi* [*P. knowlesi* hexose transporter 1 (PkHT1)] and the rodent malaria parasite *P. yoelii* [*P. yoelii* hexose transporter 1 (PyHT1)]. These studies in *Xenopus* oocytes will enable us to determine structure–function relationships and relate results to extensive knowledge about mammalian orthologues. Furthermore, the comparative functional analysis presented here is essential to the design of cross-species transfection studies because

Abbreviations used: DOG, deoxy-D-glucose; OMG, *O*-methyl-D-glucose; PfHT1, *Plasmodium falciparum* hexose transporter 1; PkHT1, *Plasmodium knowlesi* hexose transporter 1; PvHT1, *Plasmodium vivax* hexose transporter 1; PyHT1, *Plasmodium yoelii* hexose transporter 1; TgGT1, *Toxoplasma gondii* glucose transporter 1.

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interpretation of results from such studies will require knowledge of physiological characteristics of the transfected transporter. Finally, development of potential inhibitors for PfHT1 will also require assays in relevant rodent and simian models of malaria, prior to phase 1 clinical testing. A fundamental hypothesis governing our studies is that functional variation between hexose transporters of apicomplexan organisms will reflect differences in the hexose-transport properties and metabolism of host cells.

MATERIALS AND METHODS

Identification and analysis of apicomplexan hexose transporter sequences

P. vivax

The full-length sequence of *P. vivax* hexose transporter 1 (PvHT1; strain Belem) was obtained by combining limited sequence information from Genomic Sequence Tag databases with sequences from a *P. vivax* genomic DNA library kindly supplied by Dr J. Dame (University of Florida, Gainesville, FL, U.S.A.). Searching available genomic sequence tags with PfHT1 sequence using TBLASTN identified three *P. vivax* clones (207PvF05, 228PvH10 and 211PvH02) encoding amino acid residues equivalent to postions 1–58, 288–488 and 288–493 in PfHT1. The complete open reading frame for PvHT1 was constructed after sequencing clones 207PvF05 and 211PvH02.

P. knowlesi and *P. yoelii*

Sequences for PkHT1 (H strain) and PyHT1 (strain 17X NL, clone 1.1) were obtained from their respective databases (http://www.ncbi.nlm.nih.gov/projects/Malaria/).

T. gondii

TgGT1 cDNA was constructed by initially identifying a short EST sequence with homology to the mammalian facilitative hexose transporter GLUT1 and cloned by 5′- and 3′-rapid amplification of cDNA ends (RACE)-PCR (T. T. Stedman, unpublished work). The sequence was confirmed by examination of cDNA clones. Coding sequence was identical to genomic sequence fragments that have since appeared in ToxoDB, the Toxoplasma Genome Resource (http://toxodb.org/).

Derived amino acid sequences were aligned using ClustalW (MacVector version 7.1; Accelrys, San Diego, CA, U.S.A.), nearest-neighbour phylogenetic trees were constructed with Lasergene (version 4.1; DNAStar, Madison, WI, U.S.A.) and bootstrapping used MacVector. Helical assignments were made with Tmpred [8]. Accession numbers were as follows: PfHT1, AJ131457; PkHT1, AJ488937; PyHT1, AJ488938; PvHT1, AJ488939; and TgGT1, AF518411.

Cloning

P. knowlesi and *P. yoelii* genomic DNA were used as templates in PCRs to generate full-length clones for expression studies. A vector (pBSK) containing TgGT1 cDNA was used as a template in PCR to generate a full-length clone for TgGT1. Primers containing *Bgl*II restriction sites, and for the 5′ end a strong eukaryotic Kozak consensus sequence [7] before the start codon (underlined; CACCATG), were designed for each apicomplexan hexose transporter. All amplifications were for 32 cycles and as follows: 30 s at 94 °C, 45 s at 53 °C and 3 min at 68 °C using platinum *Pfx* DNA polymerase (Invitrogen, Paisley, U.K.). PCR products were ligated into pSPGT1 which contains 5′- and 3′-untranslated *Xenopus* β-globin sequences as described previously [6]. All products were verified by sequence analysis.

Heterologous expression in *Xenopus* oocytes

Xenopus oocytes were assayed as described previously in detail [6]. cRNAs for each transporter were transcribed (MEGAscript™ SP6; Ambion, Austin, TX, U.S.A.) from *Xba*I-linearized plasmids and microinjected into oocytes ($\approx$ 10 ng/oocyte). RNase-free water-injected oocytes acted as controls. Uptake assays were performed at room temperature for 20 min, 24–48 h after injection, on groups of eight oocytes in Barth's medium containing permeant D-[U-^{14}C]glucose (310 mCi · mmol^{-1}) or D-[U-^{14}C]fructose (289 mCi · mmol^{-1}) from Amersham Biosciences, Little Chalfont, Bucks., U.K. All uptake rates were linear for the times used in these assays and each result was confirmed by at least three independent experiments. Estimations of kinetic parameters by non-linear regression analysis used a Michaelis–Menten model (Prism version 3; Graphpad Software, San Diego, CA, U.S.A.). Assays to assess temperature dependency of hexose transport were performed over 20 min at specified temperatures using otherwise identical conditions to standard uptake assays.

Competition by hexose analogues was studied 24–48 h after injection in Barth's medium containing radiolabelled D-glucose (2.69 μM, 323 mCi · mmol^{-1} D-[U-^{14}C]glucose) and 35 μM unlabelled D-glucose for studies with *Plasmodium* transporters, and 3.3 μM labelled D-glucose with no constant amount of accompanying unlabelled D-glucose for studies with TgGT1, with varying amounts of competitor (all from Sigma). All solutions were allowed to equilibrate for at least 30 min prior to competition experiments, except for dehydroascorbic acid which was assayed within 15 min to minimize loss through reduction [7]. A one-site competition model was fitted to results using Graphpad Prism. Statistical comparisons between groups were carried out with Student's t test or multivariate ANOVA as appropriate.

RESULTS

Sequence analysis

Figure 1(A) displays amino acid alignments of hexose transporters from *Plasmodium* spp. that infect rodents, simians and humans, as well as from *Toxoplasma* and the major human hexose transporter (GLUT1). Predicted transmembrane segments (with helical assignments indicated as lines in Figure 1A) are well conserved in all these transporters. All apicomplexan hexose transporter sequences also contain conserved residues and motifs essential for exofacial or endofacial ligand binding, or substrate transport. These residues include tryptophan (see Figure 1A, the W residue asterisked in helix X) involved in binding to cytochalasin B, GRR/K motifs between helices II and III, and VIII and IX, a conserved glutamine residue in helix V that participates in exofacial ligand binding and/or substrate selectivity, double glutamine motif in helix VII, a proline residue in helix X and a tryptophan residue in helix XI [9].

PvHT1, PkHT1 and PyHT1 are highly similar to PfHT1 with $>$ 70% identity between all of these amino acid sequences (Figure 1B). The most closely related *Plasmodium* transporters are PvHT1 and PkHT1 (84.9% identity). The most divergent regions are located at the N-terminus, in helix XII, and at exofacial sites between helices I and II, V and VI, and IX and X.

In contrast, there is markedly more divergence between apicomplexan sequences of different genera (*Toxoplasma* versus *Plasmodium*; Figure 1B). However, the 37.2% identity between PfHT1 and TgGT1 sequences is higher than the 28.1% identity shared by PfHT1 and mammalian GLUT1. For comparative

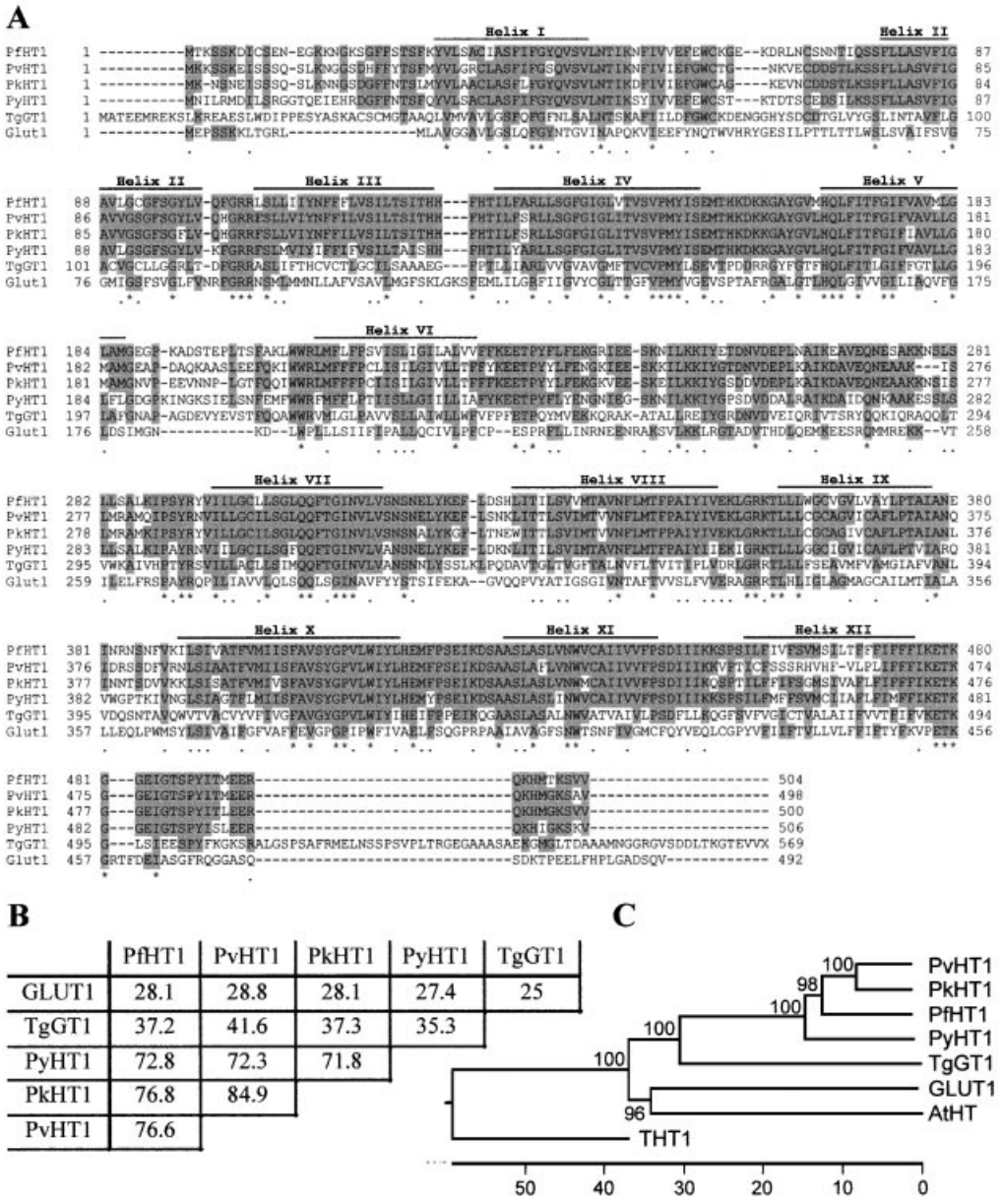

Figure 1 Sequence alignment and analysis of different apicomplexan hexose transporters

(**A**) Alignment of amino acid sequences of hexose transporters from *P. falciparum* (PfHT1), *P. vivax* (PvHT1), *P. knowlesi* (PkHT1), *P. yoelii* (PyHT1) and *T. gondii* (TgGT1) generated by the ClustalW program. Mammalian GLUT1 is also shown for comparison. Residues conserved between more than three sequences are highlighted in grey. Asterisks below the text indicate identical or conserved residues in all sequences; dots indicate semi-conserved substitutions. Assignment of helices is based on predictions made for PfHT1. (**B**) Percentage identity shared among amino acid sequences of hexose transporters shown in the alignment. (**C**) Dendrogram showing relationships between amino acid sequences of apicomplexan hexose transporters and transporters from other representative organisms. Branch lengths are proportional to sequence distances in arbitrary units. Bootstrap values are given as percentages over 1000 replications (if $> 70\%$). GLUT1, human hexose transporter 1 (accession no. NP_006507); THT1, *Trypanosoma brucei* hexose transporter 1, bloodstream form (AAA92489); AtHT, *Arabidopsis thaliana* hexose transporter (AAF74569).

purposes, only 21.4% amino acid identity is shared between the nucleoside transporter of *P. falciparum* (accession no. AAF67613) and the adenosine transporter of *Toxoplasma* (AAF03247). 40.8% identity is found between the hexokinase of *P. falciparum* (BAB55664) and that of *T. gondii* (A48457). Finally, 48.9% amino acid identity is found between *P. falciparum* (AAA29633) and *T. gondii* (AAC43443) lactacte dehydrogenase.

Detailed phylogenetic examination still positions TgGT1 more closely with *Plasmodium* transporters when compared with others (Figure 1C). *Plasmodium* and *Toxoplasma* hexose transporters therefore define a specific apicomplexan branch distinct from transporters of animals, plants and, particularly, another unicellular parasite, the kinetoplastid *Trypanosoma brucei*.

The *T. gondii* sequence is considerably longer than others (by ≈ 65 amino acids or 13%) primarily due to an extended C-terminal region, and a shorter extension at the N-terminus. Helices V, VII and X are well conserved in apicomplexan organisms, although there is variability in a helix VII triplet motif (SGL in PfHT1, PvHT1 and PkHT1, SGF in PyHT1, SIM

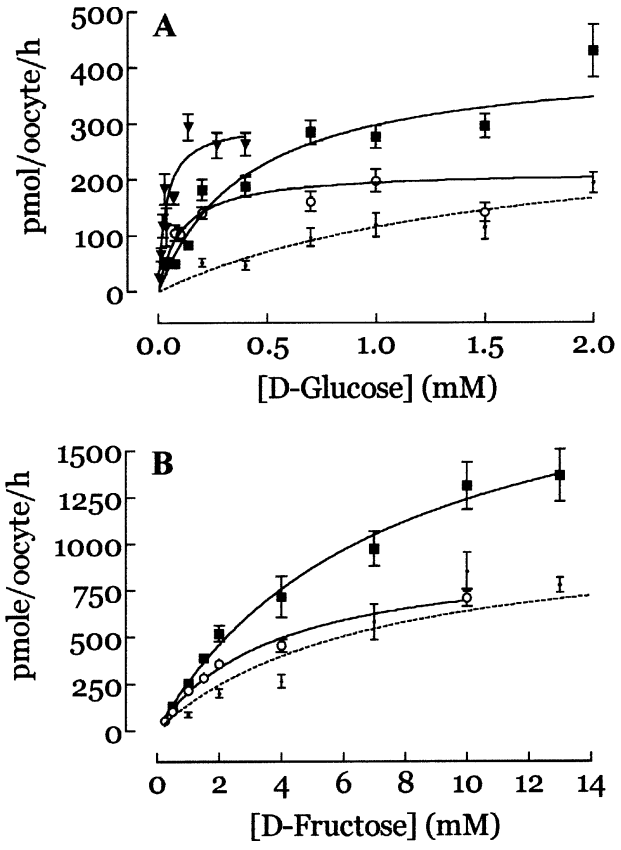

Figure 2 Hexose transport by PkHT1, PyHT1 and TgGT1 in *X. laevis* oocytes

Initial mean uptake rates (eight oocytes/concentration; means ± S.E.M.) of D-glucose (**A**) and D-fructose (**B**) are shown against concentration of substrate (representative of three experiments; see Table 1). ■, PkHT1; ○, PyHT1; ▼, TgGT1; PfHT1, reported previously [6,7], is also included for comparison (dotted lines).

Table 1 K_m studies on apicomplexan hexose transporters

Values are means ± S.E.M. from three independent replicates. K_m values for PfHT1 have been reported previously [7].

Substrate	K_m (mM) PfHT1	PkHT1	PyHT1	TgGT1
D-Glucose	0.97 ± 0.34	0.67 ± 0.16	0.12 ± 0.04	0.03 ± 0.006
D-Fructose	11.6 ± 2.75	5.12 ± 1.7	2.94 ± 0.46	Not available

in TgGT1 and QLS in GLUT1) that is important in determining substrate selectivity in mammalian transporters [10,11].

Functional characterization of apicomplexan hexose transporters

We investigated hexose transport by PkHT1, PyHT1 and TgGT1 in *Xenopus* oocytes. cRNAs encoding these transporters induced large increases in D-glucose uptake, typically 20-fold compared with water-injected controls. Results from a representative experiment assessing substrate saturation are shown in Figure 2, with published data from PfHT1 included to allow direct comparisons [7]. All *Plasmodium* transporters mediate uptake of D-fructose as well as D-glucose. Table 1 presents K_m values for these substrates. No pH dependency for glucose uptake was measured for any of these transporters (results not shown).

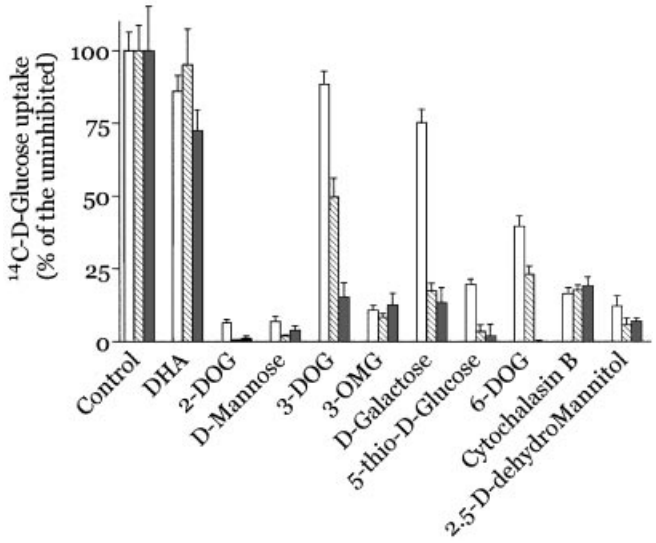

Figure 3 Active analogue studies on apicomplexan hexose transporters

Uptake assays (means + S.E.M. of eight oocytes/condition) were carried out with D-[^{14}C]glucose. The percentage D-glucose uptake for each condition was compared with uptake in uncompeted oocytes (control). DHA, dehydroascorbic acid; DOG, deoxy-D-Glucose; 3-OMG, 3-*O*-methyl-D-glucose. All compounds were used at 10 mM except cytochalasin B (50 μM). White bars, PkHT1; hatched bars, PyHT1; grey bars, TgGT1.

The K_m values for glucose for all apicomplexan transporters are different, with *T. gondii* having the highest-affinity transporter (30-fold higher affinity than PfHT1). The K_m value for glucose for PfHT1 differs significantly from PyHT1 ($P = 0.05$) and TgGT1 ($P = 0.032$). For *Plasmodium* spp. the largest difference in K_m values for glucose is between PyHT1 and PfHT1 ($\approx$ 9-fold). Similarly, K_m values for fructose also differ significantly between PfHT1 and PyHT1 ($P = 0.041$).

The specificity of a transporter for D-glucose and D-fructose (defined as V_{max}/K_m) can be determined if uptake studies for both substrates are carried out on the same batch of oocytes [7]. Results from such studies give $\approx$ 5- and $\approx$ 15-fold higher substrate specificities for glucose compared with fructose for PkHT1 and PyHT1. This compares with a 5-fold higher specificity for glucose over fructose for PfHT1, as previously reported [7].

Substrate analogue studies on apicomplexan hexose transporters

Structural requirements for interactions between apicomplexan hexose transporters and hexoses can be 'fingerprinted' using a variety of competitors and inhibitors (Figure 3) [7,12]. As predicted from the primary sequences of all three hexose transporters (Figure 1), they are inhibited by cytochalasin B.

2-Deoxy-D-glucose (2-DOG) and D-mannose inhibit D-glucose uptake in all three apicomplexan hexose transporters, suggesting that hydroxyl groups in C-2 positions are of minor importance in high-affinity interactions with these transporters. Similarly, D-glucose uptake is largely inhibited by 5-thio-D-glucose, showing the relatively minor importance of hydroxyl groups in the C-5 position for substrate–transporter interaction. Hydroxyl groups in the C-3, C-4 and C-6 positions in glucose are important for interaction with PkHT1 and PyHT1 as an excess of 3-DOG, 6-DOG or D-galactose (the 4-epimer of glucose) competes relatively poorly with glucose. In contrast, for TgGT1, whereas C-3 and

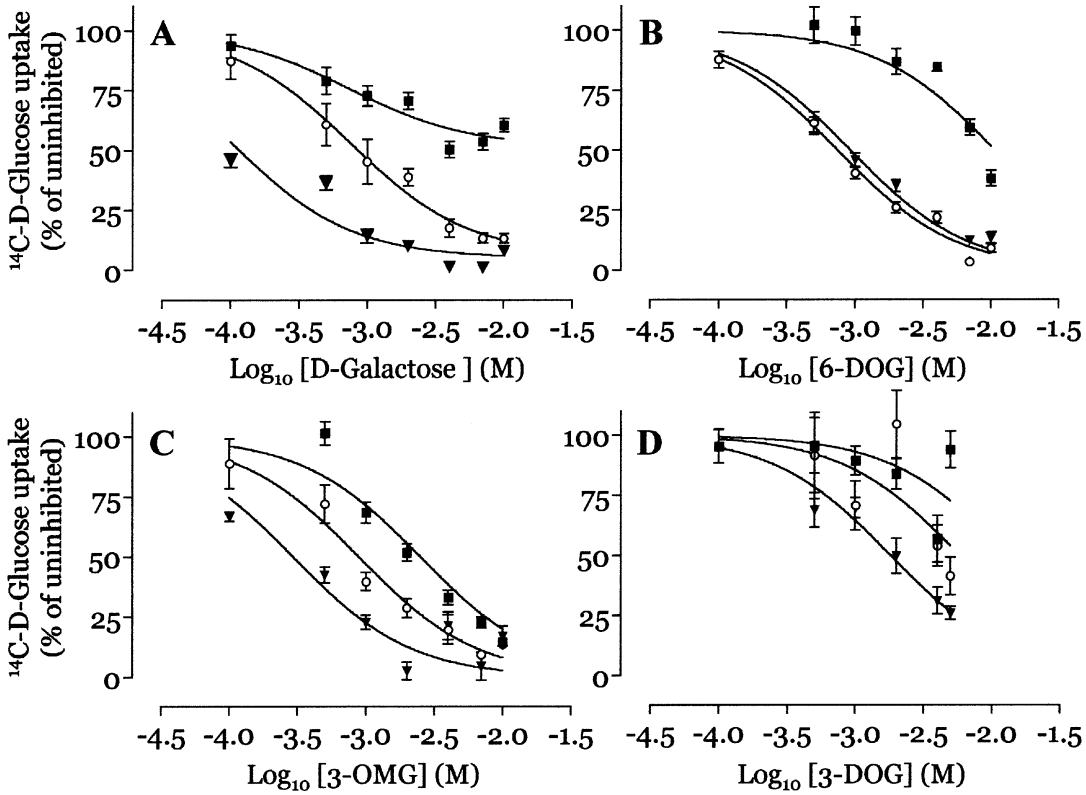

Figure 4 Competitor profiles of analogues inhibiting glucose uptake by apicomplexan transporters

Initial mean-uptake rates (eight oocytes/concentration; means ± S.E.M.) of D-glucose are plotted against the indicated concentrations of the following analogues: D-galactose (**A**), 6-DOG (**B**), 3-OMG (**C**) and 6-DOG (**D**). Each panel shows one of three experiments that were performed (see Table 2). ■, PkHT1; ○, PyHT1; ▼, TgGT1.

Table 2 Active analogue K_i studies on apicomplexan hexose transporters

Values are given as means ± S.E.M. from three independent replicates based on inhibition of D-glucose uptake. K_i values of active analogues for each transporter were compared with K_m values obtained with D-glucose and this ratio (K_i/K_m) is noted in parentheses. K_i values for PfHT1 have been reported previously [7].

Active analogue	K_i (mM) PfHT1	PkHT1	PyHT1	TgGT1
3-DOG	15 (15)	> 10* (>15)	4.11 ± 0.79 (35.1)	2.41 ± 0.48 (80.2)
3-OMG	2.05 (2)	1.85 ± 0.4 (2.76)	0.90 ± 0.1 (7.7)	0.57 ± 0.14 (18.9)
D-Galactose	7 (7)	> 10* (>15)	1.67 ± 0.43 (14.2)	0.31 ± 0.09 (10.3)
6-DOG	2.2 ± 0.9 (2.2)	9.48 ± 2.7 (14.1)	1.32 ± 0.29 (11.3)	0.91 ± 0.34 (30.3)

* Competitors (10 mM) did not inhibit more than 25% of D-glucose uptake.

C-4 positions contribute to ligand–substrate interactions, the C-6 position seems to be less important as 6-DOG inhibits D-glucose uptake. These apparent differences in properties of apicomplexan transporters, observed with an excess of competitor, suggested more detailed examination of competitor–transporter interactions.

We have focused on hydroxyl groups in the C-3, C-4 and C-6 positions, glucose derivatives in these positions giving the most divergent 'fingerprints' between apicomplexan hexose transporters. We measured K_i values for these glucose analogues (Figure 4 and Table 2) and found that the K_i for 3-DOG in all apicomplexan transporters is much higher compared with the K_m for glucose (range 15–80-fold), showing that the hydroxyl group at C-3 is involved in high-affinity interactions with these transporters. In comparison with D-glucose, small increases in K_i (4-fold or less) are observed with 3-*O*-methyl-D-glucose (3-OMG) for PfHT1 and PkHT1, showing that there is sufficient space in the ligand-binding pocket of these transporters to accommodate the bulky C-3 methyl group. This is not the case for PyHT1 and TgGT1, where increases in K_i for 3-OMG are > 7-fold compared with K_m values for glucose, although the absolute K_i values are still relatively low (< 1 mM).

All apicomplexan hexose transporters show increases in K_i values (≥ 7-fold compared with the K_m for glucose) when D-galactose, the C-4 epimer of glucose, is examined as a competitor. The C-6 position is more important for interaction with ligands in TgGT1 (K_i for 6-DOG is > 30-fold greater than the K_m for glucose) than in PkHT1 and PyHT1 (K_i for 6-DOG is ≈ 12-fold

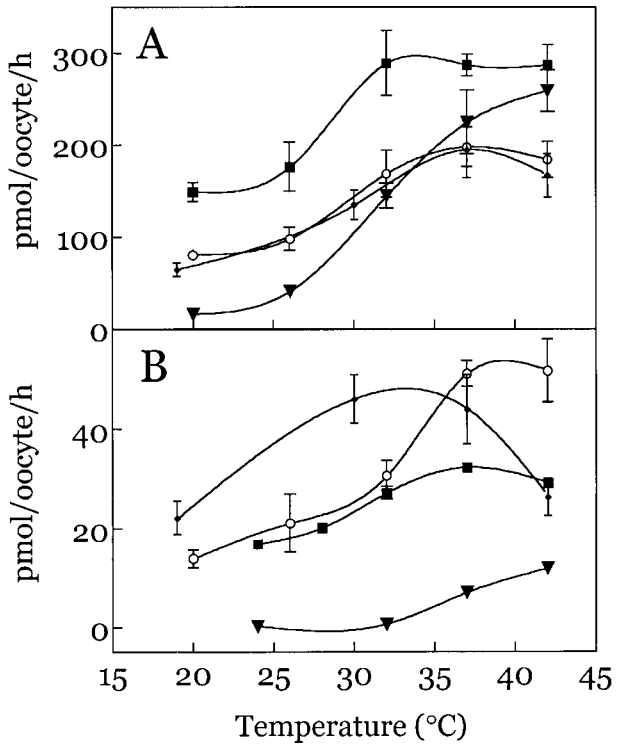

Figure 5 Temperature dependence of apicomplexan hexose transporter activity

(**A**) D-[^{14}C]Glucose uptake. (**B**) D-[^{14}C]Fructose uptake. Uptake for each temperature (means ± S.E.M. of eight oocytes/condition) was corrected for uptake in water-injected oocytes. ■, PkHT1; ○, PyHT1; ▼, TgGT1; ◆, PfHT1.

greater than the K_m for glucose). For PfHT1, interaction with C-6 appears to be less important than for the other apicomplexan transporters.

Temperature optima for hexose uptake

The temperature dependence of glucose and fructose uptake mediated by apicomplexan transporters is shown in Figure 5. All *Plasmodium* hexose transporters show a broad range of relatively high activity between 32 and 42 °C with significant (≈ 50 % of maximum) activity retained at 20 °C. Q_{10} values (the fold change in activity for a 10 °C change in temperature) for both glucose and fructose are ≈ 1.5–2.5 (for the range 25–32 °C). In contrast, the glucose-transport activity of TgGT1 is more highly temperature dependent, with a Q_{10} value of 7 (between 32 and 37 °C), and very little uptake at 20 °C. Moreover, fructose is not transported by TgGT1 when assessed at 24 °C while significant activity is measured at higher temperatures (⩾ 37 °C).

DISCUSSION

Glucose is essential for viability of asexual stages of infection with *Plasmodium* as well as for *Toxoplasma*. This study demonstrates that the transporters delivering glucose to apicomplexan intracellular pathogens differ significantly, depending on the cellular localization and host range of these parasites.

Previous phylogenetic studies based on rRNA or cytochrome *b* gene sequence comparisons placed *P. falciparum* in a different major branch from *P. vivax*/*P. knowlesi* and *P. yoelii* [13,14]. In contrast, the three primate hexose transporters PvHT1, PkHT1 and PfHT1 are the most closely related sequences we have studied. Interestingly, PkHT1 and PfHT1 also share the closest physiological characteristics (PvHT1 was not assayed); for example, the most similar K_m values for glucose and fructose transport (Table 1). The calculated glucose specificity was ≈ 5-fold higher than that of fructose for both PkHT1 and PfHT1, which is consistent with the similarity of function for these proteins, whereas the glucose specificity was much higher for PyHT1 (≈ 15-fold higher than that of fructose). Adaptation of *P. knowlesi* to the intra-erythrocytic microenvironment of *Macaca fascicularis* (the natural host) [15] and related experimental models (*M. mulatta*) may have selected for convergence of function with PfHT1, as rhesus monkeys and humans are genetically similar (and also share many transport properties of their erythrocytes when comparative studies have been done). This similarity in erythrocyte microenvironments is confirmed by the observation that *P. knowlesi* can cause human disease in spite of the phylogenetically divergent origins of PkHT1 and PfHT1 [16].

However, subtle differences between PkHT1 and PfHT1 are still discernable using functional 'fingerprinting' approaches. Initially, we screened for effects of competitors/inhibitors on apicomplexan transporters by using compounds that examine systematically each C position in glucose (Figure 3) [7]. We then defined in greater detail K_i values for competitors of D-glucose uptake that showed differences in substrate–transporter interactions between apicomplexan hexose transporters (e.g. C-4 and C-6 positions, revealed by using D-galactose and 6-DOG; see Table 2, compare PfHT1 and PkHT1).

Comparing primate malaria transporters with PyHT1, the C-3 position is less important in interactions between substrate and PyHT1 [compare ratios for $K_{i(analogue)}/K_{m(glucose)}$ for 3-DOG and 3-OMG; Table 2]. These functional differences may reflect adaptation of the rodent hexose transporter to erythrocytes that have significantly reduced capability for hexose delivery via host mammalian hexose transporters (GLUT proteins). This is because there are fewer GLUT molecules (200–400-fold) in uninfected erythrocyte membranes and lower V_{max} values for glucose in rat erythrocytes compared with human cells [17,18]. Increases in glucose utilization due to infection of rodent erythrocytes may depend on hexose delivery to parasites through new permeation pathways as well as via endogenous GLUTs, in contrast to observations made in *P. falciparum*-infected human erythrocytes where such delivery takes place predominantly through endogenous GLUTs [19].

Early studies suggested that glucose uptake by *P. yoelii* in mouse erythrocytes may be mediated by proton symport [20]. Our findings on lack of pH dependency for glucose uptake in *Xenopus* oocytes do not confirm these suggestions. These discrepancies between experimental systems are probably a result of measurements with 2-DOG, which is metabolized after transport and may subsequently interfere with transport assays, as was shown for *P. falciparum*-infected erythrocytes [19].

T. gondii multiplies in a wide range of nucleated cells, in contrast to *Plasmodium* spp. In common with other phylogenetically distinct parasitic protozoa such as *Leishmania* spp. and *Trypanasoma cruzi*, *T. gondii* has a significantly higher affinity for glucose (30 μM, Table 2) than the more closely related hexose transporters of *Plasmodium* spp. [4]. This higher affinity for glucose of TgGT1 may therefore reflect an adaptation to a relatively glucose-poor intracellular environment, particularly as nucleated host cells have large demands for glucose themselves, compared with erythrocytes. There are important additional functional differences that have emerged from our

comparisons. Interestingly, for this high-affinity transporter, as for the *Trypanasoma cruzi* kinetoplastid hexose transporter, the C-6 position is relatively more important in substrate–transporter interactions (Table 2), compared with apicomplexan relatives [21]. Furthermore, accommodation of a bulky methyl group in the C-3 position of glucose (3-OMG) is significantly less well tolerated compared with *Plasmodium* transporters [7], whereas loss of a hydroxyl group in this position gives comparable results with all apicomplexans. TgGT1, like the other apicomplexan hexose transporters, contains the consensus motif in helix 10 that confers susceptibility to inhibition by cytochalasin B. As predicted, all four transporters are inhibited to a similar extent by an excess of this compound (50 μM; Figure 3).

Other differences also emerge between TgGT1 and apicomplexan hexose transporters. Fructose transport is minimal under standard assay conditions (24 °C). At higher temperatures of assay (37–42 °C), fructose is transported by TgGT1, confirming that fructose transport capability is shared between apicomplexan transporters. To explore the possibility that this may reflect specialization of function related to host organisms, we also examined the temperature dependence of hexose uptake in all apicomplexan transporters. A broader temperature optimum may be expected for the *Plasmodium* transporters compared with TgGT1 because the *Plasmodium* transporters may function in insect stages of development which take place optimally at 22–26 °C. This contrasts with *Toxoplasma*, which is an obligate intracellular parasite confined to mammalian hosts unless it is latent in the bradyzoite form. Figure 5 confirms these predictions as the Q_{10} value for hexose transport by TgGT1 (> 7) is much higher than for hexose transport by *Plasmodium* spp. (1.5–2.5). This much higher Q_{10} value for TgGT1 is consistent with Q_{10} values obtained in other highly temperature-sensitive (and temperature-sensing; Q_{10} values > 7) ion channels studied in other systems [22], and raises the possibility that TgGT1 may also act as an environmental sensor.

Our detailed assay of function of apicomplexan hexose transporters provides new insights into their comparative biology. For example, convergence of function of PfHT1 and PkHT1 transcends phylogenetic divergence predicted from comparisons of rRNA, which would not be expected to reflect adaptation to a particular intra-erythrocytic environment. These comparative 'experiments of nature' also point to key residues responsible for functional differences, such as variations in affinity for glucose, or increased temperature sensitivity. Finally, the critical opportunity to rationally develop hexose transporter inhibitors can now be exploited fully by the use of animal models and techniques such as transfection.

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