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Trypanosome glucose transporters

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1. Introduction

The genus *Trypanosoma* comprises a group of parasitic protozoa which cause widespread disease in man and animals. African trypansomes are transmitted by tsetse flies, or other biting insects, or by coitus in the case of the equine parasite *T. equiperdum*. The South American trypanosome, infectious to humans, *T. cruzi* is transmitted fecally by reduviid bugs. The different trypanosome species have different life cycles. African trypanosomes live free in the blood, central nervous system and other tissue fluids of their mammalian hosts. *T. cruzi*, by contrast adopts an intracellular environment within their mammalian hosts. Invasion and passage through different anatomical locations within insect vectors also distinguishes the parasites.

All trypanosome species use glucose as a crucial source of energy, and all have specific plasma membrane transporters to facilitate the uptake of this molecule. Four different trypanosome glucose transporter genes have been cloned, and their function verified by expression in either *Xenopus* oocytes or Chinese hamster ovary (CHO) cells. It appears that the transporters have adapted to best suit the needs of the parasites in the different environments with which they are confronted. For example, availability of free glucose in serum differs greatly from that in a mammalian cell's cytoplasm or within the midgut of insect vectors.

The trypanosome glucose transporters belong to the glucose transporter superfamily, exemplified by the mammalian erythrocyte transporter, GLUT1 [1]. Nevertheless they have particular structural and functional features which distinguish them from their mammalian counterparts. These include: a conserved array of cysteine residues in the first exofacial loop; a relative insensitivity to various pharmacological reagents

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such as cytochalasin B; and a substrate recognition profile which includes the ability to transport D-fructose. It is possible that the trypanosome glucose transporters may represent either chemotherapeutic targets themselves, or gateways which allow the targeting of other toxic molecules to these parasites.

2. Cloning of the trypanosome glucose transporter genes

A gene cloned from a *T. brucei* EATRO 164 cDNA library [2] was found to have high homology to a glucose transporter gene previously isolated from the related kinetoplastid parasite *Leishmania enriettii* [3]. The gene was found to be a member of a clustered gene family in the *T. brucei brucei* genome. In fact, two separate isoforms, called THT1 and THT2 (THT = trypanosome hexose transporter) were isolated [4], differing predominantly in the carboxy terminal domain, and a cysteine rich first exofacial loop. Identical genes have been found in *T. brucei rhodesiense*, *T. b. gambiense*, *T. equiperdum* and *T. evansi* [5].

The *T. brucei* THT1 gene was used to probe a library of *T. cruzi* genomic DNA, and a related gene was identified [6], although only a single isoform (TcrHT1), also in multiple copies, could be identified in *T. cruzi*.

Having identified genes encoding glucose transporters from *T. brucei* and *T. cruzi*, and given the availability of related sequences from *Leishmania* parasites, regions of high conservation were identified. Oligonucleotides based on these motifs were designed and used to amplify related sequences from *Trypanosoma vivax* using the polymerase chain reaction [7]. Sequencing of three tandemly arranged TvHT1 copies present in the Y481 strain of *T. vivax* showed that there is only a single TvHT isoform (Bringaud et al., unpublished data).

The different trypanosome glucose transporter amino acid sequences are all highly homologous to one another (52–80% identity). The genes which flank the glucose transporter gene clusters from all three species of trypanosome are conserved and found in the same positions relative to the glucose transporter genes revealing a high conservation of genome organisation within the genus *Trypanosoma* (Bringaud et al., unpublished data).

All of the trypanosome glucose transporter genes conform to the widely held view of membrane topology of the glucose transporter superfamily in that hydrophobicity plots predict 12 transmembrane helices (Fig. 1). The sequences are aligned in Fig. 2.

2.1. T. brucei contains two glucose transporter isoforms: T. vivax and T. cruzi contain only one

The glucose transporters from all of the trypanosome species studied exist as multiple tandem copies. In *T. brucei* two different isoforms exist while *T. cruzi* and *T. vivax* contain just one. The copy number varies greatly between individual isolates in the case of *T. brucei* [8].

Northern blot analysis (Fig. 3) revealed that in *T. brucei* THT1 is the most abundantly expressed isoform in bloodstream form parasites, while THT2 is the most abundant in procyclics. THT2 levels are similar throughout the life-cycle while THT1 transcripts are some 40-fold more abundant than THT2 transcripts in bloodstream forms and absent or present at very low levels in procyclic forms [4]. The 3′ untranslated region of the THT1 gene is responsible for its differential expression, rendering transcripts stable in bloodstream but not procyclic cells [9]. The expression of two glucose transporter isoforms in *T. brucei* is reflected by the presence of different measurable kinetic properties of transport in bloodstream form and procyclic organisms. Bloodstream form cells possess a low affinity, high capacity glucose uptake system [10–13], while procyclics possess a higher affinity lower capacity system [14–17] (Table 1).

Sequence information alone was insufficient to assign functions to the putative transporters, hence all of the trypanosome glucose transporter genes have been expressed in heterologus systems, including CHO cells and *Xenopus* oocytes to test function. Expression of the THT2 isoform in CHO cells [16] (in which the endogenous trans-

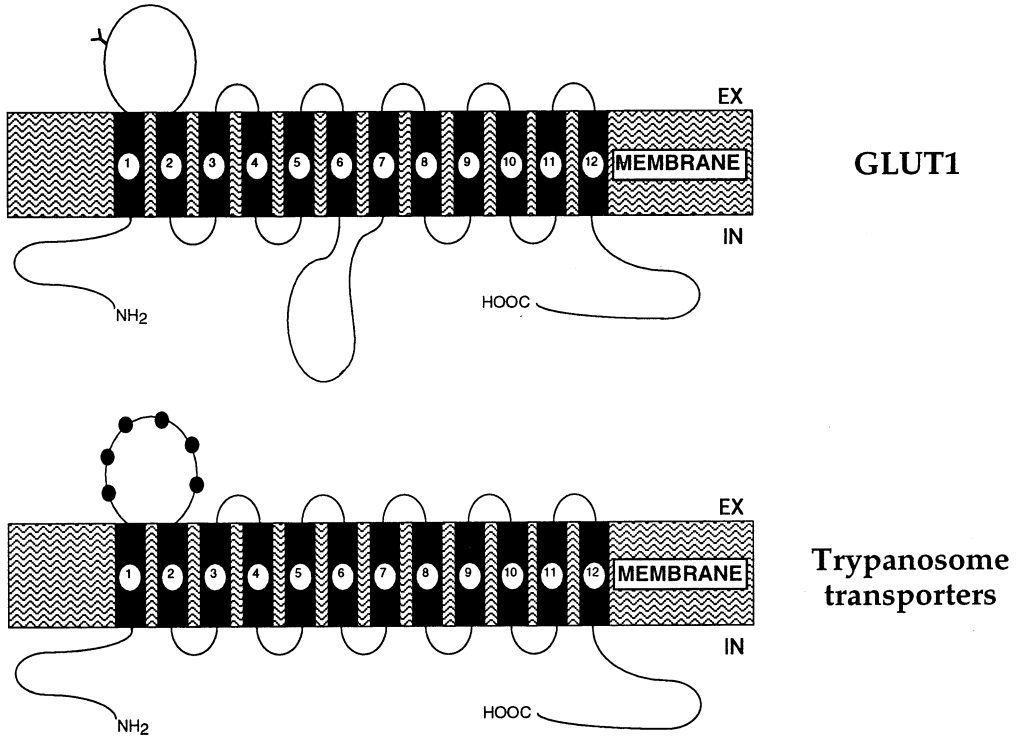

Fig. 1. Schematic representation of the mammalian GLUT1 glucose transporter and the trypanosome transporters. The 12 transmembrane hydrophobic helices are numbered. A 'Y' represents the glycoslylation site of the first exofacial loop of the mammalian transporter. Shaded circles in the trypanosomal first exofacial loop represent the cysteine residues. A particularly long intracellular loop between helices 6 and 7 of GLUT1 is not present in the trypanosome transporters.

porter was inhibited by cytochalasin B) revealed it to be kinetically and pharmacologically similar to the transporter identified in procyclic cells. THT1 cRNA, expressed in *Xenopus* oocytes [4] had a substrate recognition profile and pharmacology similar to the transporter measured in bloodstream form trypomastigotes.

The parasites therefore have two transporter genes that are expressed in a fashion which allows maximal exploitation of the host's extracellular environment. Bloodstream forms express predominantly THT1, a high capacity low affinity transporter to exploit the high concentration (~ 5 mM) of glucose in mammalian serum. Procyclic forms express the higher affinity transporter, THT2, in the insect midgut where glucose is relatively scarce, and amino acids such as proline become the major energy source [18].

T. cruzi, by contrast, has a single glucose transporter gene isoform [6], which is expressed at similar levels in epimastigotes, which dwell in the insect midgut, and trypomastigotes which live transiently in the bloodstream of the mammal before invading cells (particularly neurons and myocytes) where they transform to cytoplasmic amastigotes. Glucose transport has not been measured in amastigotes.

The metabolism of glucose by *T. cruzi* differs significantly from that of *T. brucei* bloodstream forms [19,20] which lack a functional Krebs' cycle or a full mitochondrial respiratory chain and generate, under aerobic conditions just two moles of ATP per mole of glucose consumed via the glycolytic flux which ends at pyruvate. All life-stages of *T. cruzi* have a less profligate use of glucose, using functional mitochondrial systems to generate more ATP per mole of glucose. (Procyclic

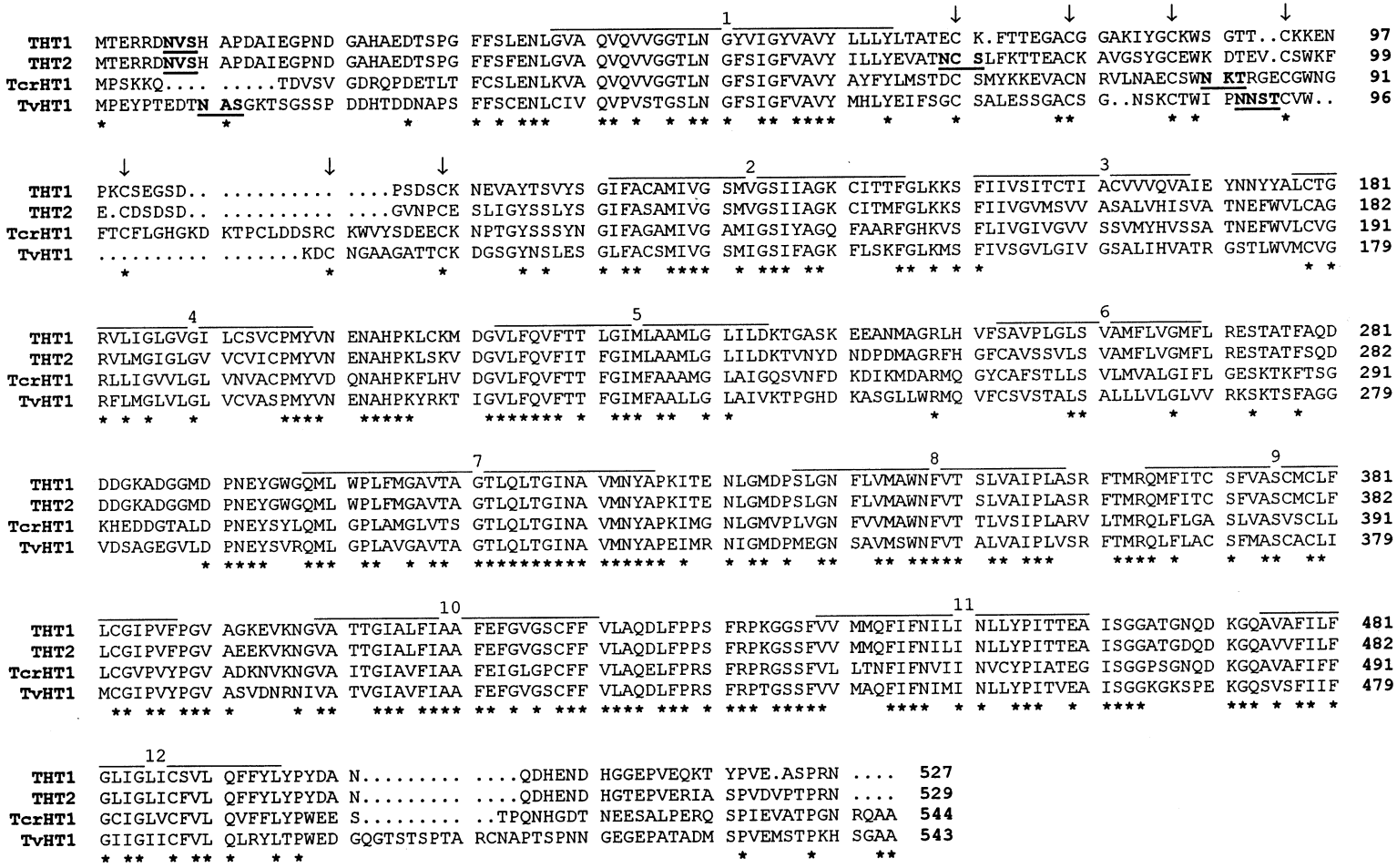

Fig. 2. Amino-acid sequence alignment of the trypanosome glucose transporters. Identical amino acids are underscored with an asterisk. The cysteines of the first exofacial loop are marked with an arrow above the first sequence. Transmembrane, hydrophobic domains are indicated. Potential *N*-linked glycosylation sites are underlined.

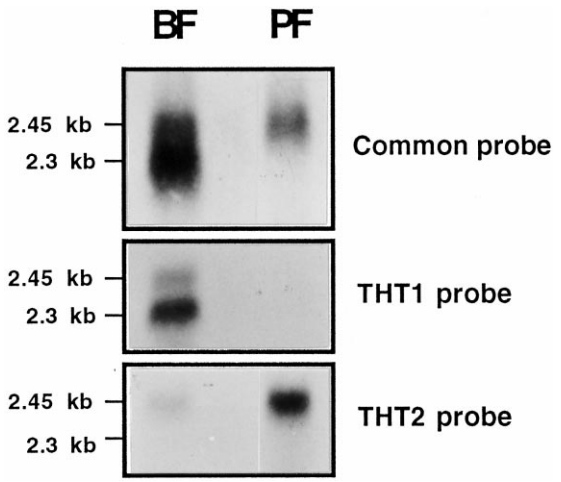

Fig. 3. Expression of the THT genes in the *T. brucei* EATRO-164 bloodstream forms (BF) and *T. brucei* Stib-247 procyclic forms (PF). The Northern blot was successively hybridized with different ^{32}P-labeled probes common to THT1 and THT2 genes (common probe), specific to THT1 genes (THT1 probe) and specific to THT2 genes (THT2 probe).

form *T. brucei* have a similar metabolism to *T. cruzi*.)

T. cruzi bloodstream form trypomastigotes are transiently bathed in the high glucose concentrations of mammalian serum, however, intracellular forms are exposed to relatively low free glucose (most cellular glucose is locked up by the host in a phosphorylated or polymeric form). Presumably for this reason they need only a single, relatively high affinity transporter (Table 1), and since bloodstream form trypomastigotes do not divide, and as their duration in the bloodstream is only transient, they have not evolved a low affinity, high capacity transporter to exploit this environment.

The metabolism of *T. vivax* is unlike that of bloodstream form *T. brucei* in that a partial Krebs' cycle is present [21,22] and glucose utilisation is more efficient in terms of ATP production. Nevertheless, it is exposed to high glucose concentrations in the mammalian bloodstream and expresses a correspondingly low affinity transporter [7] (Table 1). Intriguingly, the parasite has only a single isoform glucose transporter gene, which might reflect the fact that its passage through the tsetse fly, unlike *T. brucei*, does not take it beyond the proboscis and is relatively fast (several days as opposed to several weeks).

Table 1
Kinetic parameters and substrate specificities of the trypanosome glucose transporters

Organisms	Genes	K_m D-glucose or 2-DOG (mM)	V_{max} (nmol min^{-1} per mg protein)	Transport of D-fructose or inhibition	Type of transport
T. brucei bloodstream forms		0.49–0.9[a,b,c,d]	~250[a,b,c,d]	+[b]	Facilitated[a]
T. brucei procyclic forms		0.045–0.080[e,f,g]	4–10[e,f,g]	+[e,f]	Facilitated/ Active[e,f]
T. brucei	THT1 (*Xenopus*)	ND	ND	+[h]	Facilitated[h]
	THT2 (CHO)	0.151 (2-DOG)[f]	4.3[f]	+[f]	Facilitated[f]
T. cruzi trypomastigote forms		0.294 (2-DOG)	1.4	ND	Facilitated
T. cruzi epimastigote forms[i,j]		0.312 (2-DOG)	3.65	+	Facilitated
T. cruzi	TcrHT1 (CHO)	0.315 (2-DOG)	7.7	+	Facilitated
T. vivax bloodstream forms[k]		0.585	88.5	+	Facilitated
T. vivax	TvHT1 (CHO)	0.545 (2-DOG)	4.3	+	Facilitated

Values are from: [a] [10]; [b] [11]; [c] [13]; [d] [31]; [e] [14]; [f] [16]; [g] [17]; [h] [4]; [i] [6]; [j] [26]; [k] [7].

2.2. Facilitative versus secondary active transport

The uptake of nutrients into cells may occur by a variety of mechanisms [23]. Large polar molecules, such as glucose, and indeed most biochemical substrates are too large and polar to simply diffuse across the lipid bilayer, and thus require specific transport proteins to catalyse uptake. Cells may accumulate nutrients without energy expenditure, allowing the thermodynamic energy in a diffusion gradient to propel substrates into cells. Alternatively, if a diffusion gradient cannot be relied upon (i.e. if substrate is scarce or a cell needs to concentrate it beyond the extracellular level) energy is expended. Such active transport can be direct (the transporter uses either ATP or pyrophosphate to accumulate substrate), or indirect. Indirect active transport involves the co-transport of two substrates (either in the same, symport, or opposite, antiport, direction). The co-transport of glucose with sodium ions in the mammalian intestine, and lactose with protons in *Escherichia coli* serve as classic examples of secondary active transport [23]. The energy to drive these systems comes from the diffusion gradients of co-transported ions which are maintained by secondary energy dependent pumping systems.

The different types of transport may be distinguished experimentally. Active transporters accumulate substrates against a concentration gradient and comparison of intracellular and extracellular concentrations of substrate can show when a substrate has been actively concentrated. If a second substrate (usually an ion) is required, transport in the presence and absence of that ion will be markedly different. Finally pharmacological reagents which either dissipate the ion gradients required to drive secondary active transport, or agents which inhibit energy production within the cell can also inhibit secondary active transport.

It is generally agreed that bloodstream forms of *T. brucei* depend on a facilitiative diffusion molecule to accumulate glucose [10–13]. Given these organisms have evolved in a glucose rich environment, and that glucose is metabolised virtually instantaneously, ensuring a perpetual concentration gradient, energy expenditure on glucose accumulation would be unnecessary. One study did suggest a dependence on sodium ions at low external glucose concentration [24].

The case of the procyclic trypanosome is more controversial. Procyclics are exposed to low levels of glucose in the tsetse midgut, however, after a tsetse feed they are exposed, transiently, to high levels of the sugar in mammalian serum. The first study on these organisms revealed a relatively high affinity transporter, which according to pharmacological investigation was dependent on the co-transport of protons [14]. Both the respiratory inhibitor KCN and the protonophore FCCP reduced transport. Two further studies, both employing high concentrations of substrate failed to show either an accumulation of glucose beyond the external levels [15], or inhibition by KCN or FCCP [17]. In fact, an inhibitory effect by both KCN and FCCP depends on the concentration of substrate [16]. It appears that these reagents inhibit transport at low external glucose concentration, but exert progressively less impact as the substrate concentration rises to 1 mM. This phenomenon is characteristic of 'slippage' [25] whereby the dependence on the counter-ion is diminished as substrate concentration rises. In the case of the procyclic trypanosome, such a mechanism makes physiological sense given that normally the parasites are exposed to low external glucose and might expend energy upon its acquisition. During the transient explosion in glucose concentration following a tsetse meal, however, dependence on energy to acquire the abundant substrate would be futile. One difficulty with the 'slippage' hypothesis is that transport via THT2, the procyclic transporter, expressed in CHO cells, had no dependency on protons or energy regardless of substrate concentration [16]. The situation in procyclic cells might be more complicated, involving other phenomena including putative auxiliary factors or membrane potential which would be effected by the same pharmacological reagents used to assess proton dependence.

All available evidence suggests that *T. cruzi* [6,26] and *T. vivax* [7] have straight forward facilitative transporters, although controversy also surrounds the mechanism employed by leishmanial transporters [27,28].

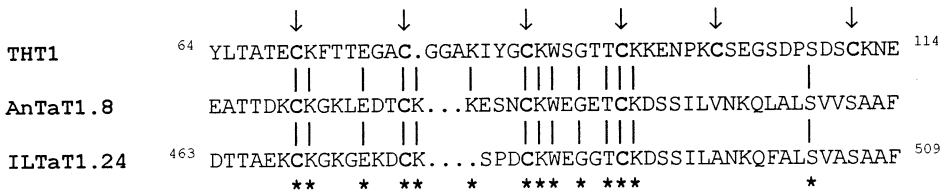

Fig. 4. Alignment of the cysteine rich loop of THT1 with domains from two *T. brucei* variant surface glycoproteins. The amino acid sequence of the first exofacial loop, when used to search the protein data bases identified homology with small conserved regions of *T. brucei* variant surface glycoproteins. Alignment with the regions of the AnTaT1.8 and ILTaT1.24 sequences is shown here. The cysteine residues are over-scored with an arrow. An asterisk below the aligned sequences highlights identical residues.

2.3. The first extracellular loop of trypanosome glucose transporters contains a conserved arrangement of cysteine residues

All of the trypanosome glucose transporters possess numerous, spatially conserved cysteine residues in their first exofacial loop. Extensive analysis of the sequences of THT1 and THT2 reveal the loop to have been inserted independently [5], probably to replace a different type of first exofacial loop in an ancestral gene. Intriguingly, when the amino-acid sequence of the loop was used to scan protein sequence data-bases it identified a similar conserved arrangement of cysteine residues in variant surface glycoproteins from African trypansomes (Fig. 4). In fact, many trypanosomatid surface proteins reveal this arrangement of cysteines [29]. The motif has been conserved suggesting it performs a critical role in the architecture of kinetoplastid surface membranes.

3. Glycosylation of trypanosomal glucose transporters

The mammalian GLUT1 transporter, and various other eukaryotic hexose transporters are glycosylated, usually in the first exofacial loop [30] which corresponds to the cysteine rich loop of the trypanosome transporters described above. Western blot analysis of THT1 after proteinase K digest of intact parasites, using site-specific antibodies against the amino terminus, carboxy terminus or two predicted extracellular loops connecting helices 5/6 and 7/8, has revealed that THT1 is oriented in the plasma membrane according to the predicted model based on GLUT1 (Seyfang et al., unpublished data).

Amino acid analysis of the various trypanosomal glucose transporters revealed significant differences in the occurrence of potential *N*-linked glycosylation sites (Fig. 2). Asn-69 in *T. brucei* THT2, Asn-81 in *T. cruzi* TcrHT1 and Asn-90, Asn-91 in *T. vivax* TvHT1 are the only potential exofacial *N*-glycosylation sites in these transporters, all located within the first extracellular loop.

The *T. brucei* bloodstream form transporter, THT1, lacks potential *N*-linked glycosylation sites on any of the predicted extracellular loops (only the loops which end up outside the cell are topologically available for glycoslylation within the endoplasmic reticulum). THT1 does contain one *N*-glycosylation consensus sequence (Asn-7), although this is located on the amino terminal tail and is predicted to face the cytoplasm. THT2 (Asn-7) and TvHT1 (Asn-10) also have additional *N*-glycosylation consensus sequences at similar positions.

De-glycosylation experiments with THT1-enriched plasma-membrane fractions [31] (using *N*-glycosidase, *O*-glycosidase and sialidase treatment) in combination with Western blot analysis showed no band shift (reduced molecular weight) for the THT1 protein [32]. In contrast, the GLUT1 protein purified from human erythrocytes and de-glycosylated had a reduction in apparent molecular weight of about 3 kDa after *N*-glycosidase F treatment. THT1-enriched plasma-membrane fractions were also analysed for glycoproteins by lectin blot analysis. A glyco-

protein of 45 kDa (gp45) which co-migrated on reducing SDS-PAGE gels with THT1 [32] was detected. However, in non-denaturing gels, THT1 showed a lower electrophoretic mobility (shifting from 45 to 51 kDa) and under these conditions no glycoproteins co-migrated with THT1. These cumulative data demonstrated that THT1 is not glycosylated, in agreement with the predictions drawn from amino acid sequence data and membrane topology predictions.

It is believed that glycoslylation is critical in correctly orienting mammalian transporters in the plasma membrane [33]. Clearly in the case of bloodstream form *T. brucei* the transporter can be oriented without glycoslylation. Possibly the dense packaging of the highly glycosylated variant surface glycoproteins of bloodstream form *T. brucei* might influence the need for glycosylation on transporter proteins in the plasma membrane. Interestingly, expression of THT2, TvHT1 and TcrHT1 was successful in CHO cells, while THT1 could not be functionally expressed (Tetaud et al., unpublished data). Possibly it is the lack of glycosylation to THT1 which hinders expression in mammalian cells.

4. Therapeutic targets and therapeutic targeting

Any aspect of a parasite's biochemical make-up may be vulnerable to therapeutic attack, particularly where significant differences between host and parasite can be identified. In the case of trypanosome glucose transporters significant differences can be identified in terms of both the pharmacology and substrate recognition profiles when compared to the mammalian GLUT1 transporter. For example, all of the trypanosome transporters also carry D-fructose [7,16,26,34] while GLUT1 does not. GLUT1 is very much more susceptible to cytochalasin B and phloretin than the trypanosome transporters. An extensive study of other reagents would undoubtedly identify some which react more potently with the trypanosome transporters. None of the drugs used in the chemotherapy of human African trypanosomiasis inhibit the glucose transporter of bloodstream form trypomastigotes when used at concentrations usually toxic to the cells, suggesting that none of these drugs exert their activity at the level of this transporter (Barrett et al., unpublished observations).

Detailed analysis of substrate analogues, substituting the various hydroxyl groups has identified the key features involved in the structure-activity relationship of the various trypanosome glucose transporters (Fig. 5). Bloodstream form *T. brucei* depends exclusively on glucose metabolism to generate energy, hence inhibition of glucose uptake would certainly be expected to exert a lethal effect. So far, however, specific inhibitors of the *T. brucei* glucose transporter have not been identified. Armed with the substrate recognition information, and given the high capacity of the *T. brucei* bloodstream from glucose transporter to fulfill the prodigious appetite these cells have for this substrate, it might be possible to subvert the transporter, to carry toxic analogues of glucose into the cell.

A similar situation exists in the case of the melaminophenyl based arsenical and diamidine classes of drug which enter trypanosomes via an unusual amino-purine transporter, termed P2 [35]. The drugs share a chemical motif with the normal amino-purine substrates which is recognised by the transporter. Loss of the transporter renders the parasites resistant to these drugs.

Since the *T. brucei* bloodstream form transporter does not make hydrogen bonds with the hydroxyls at positions 2 and 6 of the glucose ring [11], these sites were considered available for the attachment of other chemical constituents, which would not interfere with recognition by the transporter. In fact, in the case of the C-6 position, a strict limit on the size of substituent groups has been noted (Barrett et al., unpublished data), whereas, provided substituent groups were neither charged nor aromatic, relatively large replacements could be added at position C-2. At least one compound containing a substituent group at position C-2 has been developed, which is toxic to bloodstream form parasites grown in vitro (Barrett et al., unpublished data). Fructose analogues have also been developed [36], and toxic examples are known. Further work is underway to assess the chemotherapeutic value of these compounds,

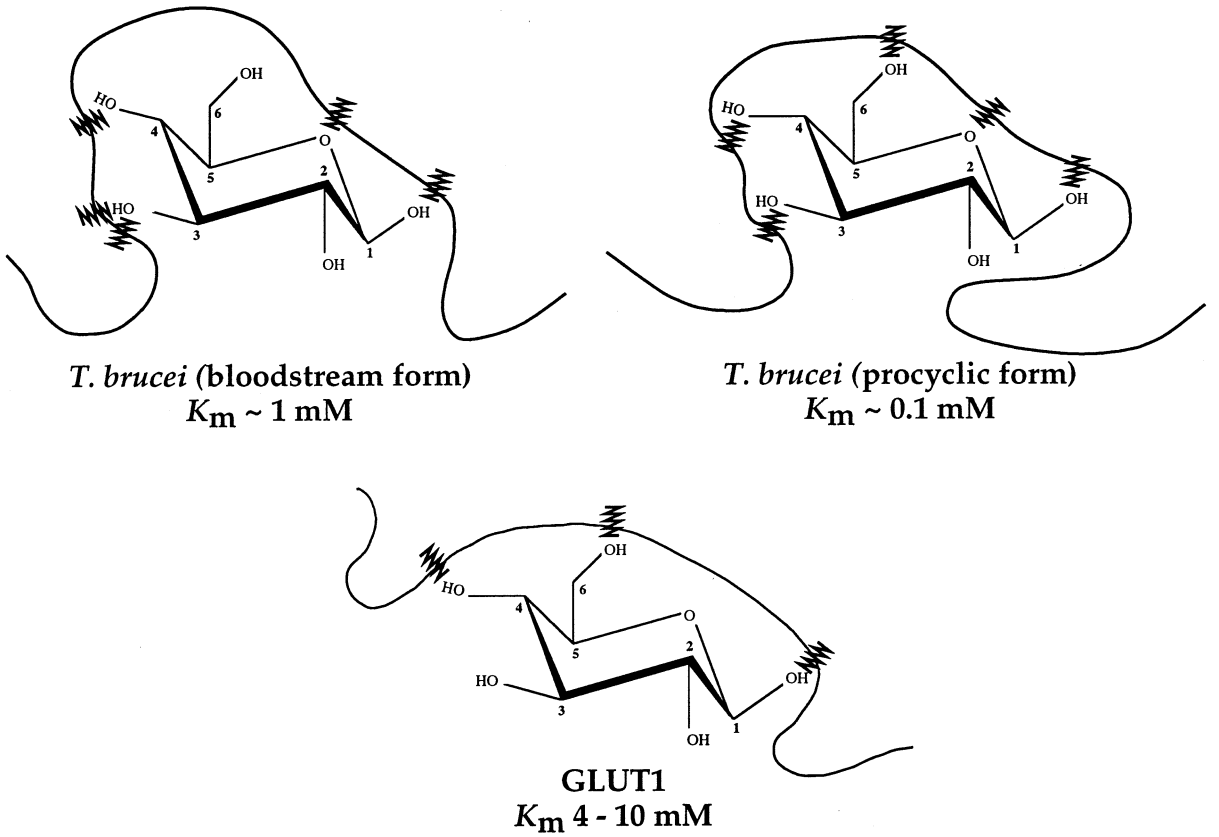

Fig. 5. Substrate recognition of the *T. brucei* bloodstream form, and procyclic form glucose transporters, compared with mammalian GLUT1. Hydrogen bonds predicted to occur between the transporters and D-glucose are represented by jagged lines. The different carbon positions are numbered 1–6 (data from [11,16,39]).

and to distinguish whether the products actually inhibit the transporter itself or hit intracellular targets once taken into the cell via the transporter. A potential drawback with the glucose transporter relates to its low affinity for substrate, and the high abundance of glucose within serum, which would out-compete analogues for the transporter if such analogues were prescribed in quantities within the range of currently used antimicrobial drugs.

5. Discussion

Glucose transporter genes have been cloned from several parasitic protozoa of the genus *Trypanosoma*. The function of the genes has been verified by expression in both *Xenopus* oocytes and CHO cells.

T. brucei contains two separate glucose transporter gene isoforms, which account for the different kinetic properties of the transporters measured in the different life cycle forms of the parasite. *T. cruzi*, and *T. vivax* contain only a single isoform. Substrate affinity and possibly mechanistic differences distinguish the transporters, in a manner which may reflect the different extracellular environments to which the parasites are exposed.

All of the trypanosome glucose transporters also recognise D-fructose which distinguishes them from the main mammalian glucose transporter, GLUT1. However, there are at least four other mammalian plasma membrane hexose

transporters expressed in different tissues, with a range of substrate specificities, including two isoforms (GLUT2-liver and GLUT5-intestine) [37,38] which also recognise D-fructose, highlighting the difficulties in pin-pointing unique features of the trypanosome transporters which might hinder their utility as chemotherapeutic targets.

The trypanosome transporters all contain a cysteine-rich first exofacial loop with a structure conserved in other trypanosomatid membrane proteins, which may play a critical role in the membrane architecture of these parasites. The comparative situation of the loop in THT1 and THT2 suggests it was inserted independently into ancestral genes. The presence of a similar structure in other trypanosomatid membrane proteins, might suggest that the insertion of 'cassettes' which donate particular functions to proteins, has been involved in the evolution of trypanosome membrane proteins.

Structure activity relationship studies between the transporters and their substrates has allowed the development of analogues which are still recognised by the transporter, and may herald a new pathway in the development of novel anti-trypanosomal reagents, which exploit the glucose transporter as a specific gateway to carry toxic reagents into the parasites.

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References

[1] Gould GW, Holman GD. The glucose transporter family: structure, function and tissue-specific expression. Biochem J 1993;295:329–41.
[2] Bringaud F, Baltz T. A potential hexose transporter gene expressed predominantly in the bloodstream form of *Trypanosoma brucei*. Mol Biochem Parasitol 1992;52:111–22.
[3] Cairns BR, Collard MW, Landfear SM. Developmentally regulated gene from *Leishmania* encodes a putative membrane transport protein. Proc Natl Acad Sci USA 1989;86:7682–6.
[4] Bringaud F, Baltz T. Differential regulation of two distinct families of glucose transporter genes in *Trypanosoma brucei*. Mol Cell Biol 1993;13:1146–54.
[5] Bringaud F, Baltz T. African trypanosome glucose transporter genes: organisation and evolution of a multigene family. Mol Biol Evol 1994;11:220–30.
[6] Tetaud E, Bringaud F, Chabas S, Barrett MP, Baltz T. Characterization of glucose transport and cloning of a hexose transporter gene in *Trypanosoma cruzi*. Proc Natl Acad Sci USA 1994;91:8278–82.
[7] Waitumbi JN, Tetaud E, Baltz T. Glucose uptake in *Trypanosoma vivax* and molecular characterization of its transporter gene. Eur J Biochem 1996;237:234–9.
[8] Barrett MP, Bringaud F, Doua F, Melville SE, Baltz T. Hypervariability in gene copy number for the glucose transporter genes in trypanosomes. J Euk Microbiol 1996;43:244–9.
[9] Hotz H-R, Lorenz P, Fischer R, Krieger S, Clayton C. Role of 3′-untranslated regions in the regulation of hexose transporter mRNAs in *Trypanosoma brucei*. Mol Biochem Parasitol 1995;75:1–14.
[10] Gruenberg J, Sharma PR, Deshusses J. D-Glucose transport in *Trypanosoma brucei*. Eur J Biochem 1978;89:461–9.
[11] Eisenthal R, Game S, Holman GD. Specificity and kinetics of hexose transport in *Trypanosoma brucei*. Biochim Biophys Acta 1989;985:81–9.
[12] Seyfang A, Duszenko M. Specificity of glucose transport in *Trypanosoma brucei*: effective inhibition by phloretin and cytochalasin B. Eur J Biochem 1991;202:191–6.
[13] Ter Kuile BH, Opperdoes FR. Glucose uptake by *Trypanosoma brucei*: rate-limiting steps in glycolysis and regulation of the glycolytic flux. J Biol Chem 1991;266:857–62.
[14] Parsons M, Nielsen B. Active transport of 2-deoxy-D-glucose in *Trypanosoma brucei* procyclic forms. Mol Biochem Parasitol 1990;42:197–204.
[15] Ter Kuile BH, Opperdoes FR. Mutual adjustment of glucose uptake and metabolism in *Trypanosoma brucei* grown in a chemostat. J Bacteriol 1992;174:1273–9.
[16] Barrett MP, Tetaud E, Seyfang A, Bringaud F, Baltz T. Functional expression and characterization of the *Trypanosoma brucei* procyclic glucose transporter, THT2. Biochem J 1995;321:687–91.
[17] Wille U, Seyfang A, Duszenko M. Glucose uptake occurs by facilitated diffusion in procyclic forms of *Trypanosoma brucei*. Eur J Biochem 1996;236:228–33.
[18] Cazzulo JJ. Aerobic fermentation of glucose by trypanosomatids. FASEB J 1992;6:3153–61.
[19] Fairlamb AH, Opperdoes FR. Carbohydrate metabolism in African trypanosomes, with special reference to the glycosome. In: Morgan MJ, editors. Carbohydrate Metabolism in Cultured Cells. New York: Plenum, 1986:183–219.

[20] Opperdoes FR. Compartmentation of carbohydrate metabolism in trypanosomes. Ann Rev Microbiol 1987;41:127–51.
[21] Gardiner PR. Recent studies of the biology of *Trypanosoma vivax*. Adv Parasitol 1989;28:229–317.
[22] Bowman IBR, Flynn IW. Oxidative metabolism of trypanosomes. In: Lumsden WHR, Evans DA, editors. Biology of the Kinetoplastida, vol. 1. New York: Academic, 1976:435–476.
[23] Stein WD. Channels, Carriers, and Pumps: An Introduction to Membrane Transport. San Diego: Academic, 1990.
[24] Munoz-Antonia T, Richards FF, Ullu E. Differences in glucose transport between bloodstream and procyclic forms of *Trypanosoma brucei* rhodesiense. Mol Biochem Parasitol 1991;47:73–82.
[25] Eddy AA. Slip and leak models of gradient-coupled solute transport. Biochem Soc Trans 1980;8:271–3.
[26] Tetaud E, Chabas S, Giroud C, Barrett MP, Baltz T. Hexose uptake in *Trypanosoma cruzi*: structure-activity relationship between substrate and transporter. Biochem J 1996;317:353–9.
[27] Zilberstein D, Dwyer DM. Protonmotive force-driven active transport of D-glucose and L-proline in the protozoan parasite *Leishmania donovani*. Proc Natl Acad Sci USA 1985;82:1716–20.
[28] Ter Kuile BH, Opperdoes FR. Uptake and turnover of glucose in *Leishmania donovani*. Mol Biochem Parasitol 1993;60:313–22.
[29] Carrington M, Boothroyd J. Implications of conserved structural motifs in disparate trypanosome surface-proteins. Mol Biochem Parasitol 1996;81:119–26.
[30] Mueckler M. Facilitative glucose transporters. Eur J Biochem 1994;219:713–25.
[31] Seyfang A, Duszenko M. Functional reconstitution of the *Trypanosoma brucei* plasma-membrane D-glucose transporter. Eur J Biochem 1993;214:593–7.
[32] Seyfang A. Characterization and isolation of the glucose transporter from *Trypanosoma brucei*. PhD thesis. University of Tübingen, 1993.
[33] Hresko RC, Kruse M, Strube M, Mueckler M. Topology of the GLUT1 glucose transporter deduced from glycosylation scanning mutagenesis. J Biol Chem 1994;269:20482–8.
[34] Fry AJ, Towner P, Holman GD, Eisenthal R. Transport of D-fructose and its analogues by *Trypanosoma brucei*. Mol Biochem Parasitol 1993;60:9–18.
[35] Carter NS, Fairlamb AH. Arsenical resistant trypanosome lack an unusual adenosine transporter. Nature 1993;361:173–5.
[36] Page P, Blonski C, Perie J. An improved chemical and enzymatic synthesis of new fructose derivatives for import studies by the glucose transporter in parasites. Tetrahedron 1996;52:1557–72.
[37] Colville CA, Seatter MJ, Jess TJ, Gould GW, Thomas HM. Kinetic analysis of the liver-type (GLUT2) and brain-type (GLUT3) glucose transporters in *Xenopus* oocytes: substrate specificities and effects of transport inhibitors. Biochem J 1993;290:701–6.
[38] Burant CF, Takeda J, Brot-Laroche E, Bell GI, Davidson NO. Fructose transporter in human spermatozoa and small intestine is GLUT5. J Biol Chem 1992;267:14523–6.
[39] Silverman M. Structure and function of hexose transporters. Annu Rev Biochem 1991;60:757–94.