REVIEW ARTICLE

Kinetoplastid glucose transporters

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Protozoa of the order kinetoplastida have colonized many habitats, and several species are important parasites of humans. Adaptation to different environments requires an associated adaptation at a cell's interface with its environment, i.e. the plasma membrane. Sugar transport by the kinetoplastida as a phylogenetically related group of organisms offers an exceptional model in which to study the ways by which the carrier proteins involved in this process may evolve to meet differing environmental challenges. Seven genes encoding proteins involved in glucose transport have been cloned from several kinetoplastid species. The transporters all belong to the glucose transporter superfamily exemplified by the mammalian erythrocyte trans-

porter GLUT1. Some species, such as the African trypanosome *Trypanosoma brucei*, which undergo a life cycle where the parasites are exposed to very different glucose concentrations in the mammalian bloodstream and tsetse-fly midgut, have evolved two different transporters to deal with this fluctuation. Other species, such as the South American trypanosome *Trypanosoma cruzi*, multiply predominantly in conditions of relative glucose deprivation (intracellularly in the mammalian host, or within the reduviid bug midgut) and have a single, relatively high-affinity type, transporter. All of the kinetoplastid transporters can also transport D-fructose, and are relatively insensitive to the classical inhibitors of GLUT1 transport cytochalasin B and phloretin.

INTRODUCTION

The simple sugar glucose, in the polymeric form cellulose, is probably the most abundant biological molecule on Earth. Together with other sugars it represents a very important nutrient for many types of cell, not only in mammals and other higher organisms but also in microbes. Glucose is sufficiently large and polar to preclude its passage through cellular membranes by simple diffusion, so cells that utilize glucose must elaborate specific transport systems within their plasma membranes to allow the acquisition and subsequent metabolism of this nutrient. During the past 10 years, DNA sequencing has yielded the primary structures of sugar transporters from numerous organisms, and expression of these genes in heterologous systems has greatly enhanced our understanding of these proteins.

The phylogenetic order known as the kinetoplastida contains single-celled flagellates, several species of which are important parasites. These include African trypanosomes, which cause sleeping sickness in humans and a number of veterinary diseases, *Leishmania* species, which cause a wide spectrum of disease world-wide, and *Trypanosoma cruzi*, which causes Chagas' disease in South America. Other species, such as *Crithidia fasciculata*, parasitize insects. The life cycle of kinetoplastids can be complex, sometimes involving numerous developmental stages in several hosts (Figure 1). The African trypanosomes, for example, are transmitted between mammalian hosts by tsetse flies, in which different metabolic requirements are imposed on the parasites. Successful adaptation of these organisms to hostile environments within their vectors and hosts depends on their ability to maintain intracellular homeostasis. The interface

between parasite and host occurs at the level of the parasite surface membrane. Consequently, studying glucose transport and glucose transporters in these parasites provides a good model of how these parasites adapt to changing environments. Moreover, such transporters potentially represent specific targets for drug and/or immunotherapy. As the kinetoplastida are a large group of phylogenetically related organisms that are subject to a range of environmental conditions, the adaptation of glucose transporters to deal with these differences provides an exceptional model for increasing our understanding of the structure/activity relationship of this group of molecules.

In this review, we summarize the present state of knowledge of the kinetoplastid glucose transporters and discuss their diversity, highlighting differences in kinetic properties and substrate specificity.

ENERGY METABOLISM IN KINETOPLASTIDS

Glucose metabolism and the glycosome

Glucose can be metabolized by either the glycolytic pathway or the pentose phosphate pathway [1], to varying degrees depending on a cell's specific requirements. Bloodstream-form *T. brucei* and other members of the subgenus trypanozoon (*T. brucei* group) depend exclusively on substrate-level phosphorylation to generate ATP. Other kinetoplastids, including the insect stages of the *T. brucei* group trypanosomes, can produce ATP from the Krebs cycle and the respiratory chain.

All members of the kinetoplastida possess organelles related to the peroxisomes found in other species, but containing the first seven enzymes of the Embden–Meyerhof–Parnas glycolytic

Abbreviations used: DCCD, *N,N'*-dicyclohexylcarbodi-imide; 2-DOG, 2-deoxy-D-glucose; FCCP, carbonyl cyanide-4-(trifluoromethoxy)-phenylhydrazone.

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Figure 1 Life cycles of parasitic kinetoplastids

(a) African trypanosomes. Procyclic forms of the parasite proliferate in the tsetse-fly midgut, then undergo several developmental stages before migrating to the salivary glands, where they differentiate into the mammalian infective metacyclic forms. These are injected in the tsetse bite and differentiate into long slender bloodstream-form trypomastigotes, which can also penetrate the central nervous system (CNS). A form referred to as 'short stumpy' may be pre-adapted for further development in the tsetse fly, as parasites are taken up in a blood meal. (b) *Leishmania* species. Promastigote forms of the parasite proliferate in the sand-fly midgut and then migrate to the proboscis, from where they are injected into the mammalian host during a sand-fly feed. Promastigotes are taken up by macrophages, and the parasites develop into amastigotes within phagolysosomes. Amastigotes, either within macrophages or free in the bloodstream, differentiate back to promastigotes when taken up in a sand-fly blood meal. (c) *Trypanosoma cruzi*. Epimastigote forms of the parasite proliferate in the gut lumen of reduviid bugs. They then move to the hind-gut and are transformed into metacyclic trypomastigotes, which are expelled in the faeces of the insect as it feeds on a mammalian host. Parasites enter the host via skin abrasions and can invade various cell types, including macrophages, heart and muscle. They differentiate into amastigotes and proliferate free in the cytosol of infected cells, then re-differentiate into trypomastigotes which are taken up by reduviids feeding on infected blood. Abbreviation: N, nucleus.

pathway, plus other enzyme systems. These organelles were, aptly, named glycosomes [2,3].

Glucose metabolism in bloodstream-form African trypanosomes

T. brucei bloodstream forms, which lack a functional Krebs cycle and respiratory chain, demonstrate qualitatively different routes of metabolism depending on the presence or absence of oxygen. Pyruvate is the exclusive end-product under aerobic conditions, whereas in anaerobiosis pyruvate and glycerol are produced in equimolar concentrations [3–5]. In aerobic conditions, the re-oxidation of NADH is performed by a mitochondrial shunt involving a trypanosome alternative oxidase and glycerol-3-phosphate dehydrogenase [5,6], and 2 mol of ATP are synthesized per mol of glucose. In anaerobic conditions, dihydroxyacetone phosphate acts as a final electron acceptor, and 1 mol of ATP is produced per mol of glucose. Other kinetoplastids use the respiratory chain to maintain the intracellular redox potential.

Glucose metabolism in procyclic-form African trypanosomes and other kinetoplastids

Procyclic-form *T. brucei* and the various developmental stages of *T. cruzi*, *Leishmania* and *Crithidia* are observed to produce high quantities of CO_2 , succinate, acetate and L-lactate under aerobic conditions. *Leishmania* offers an exception in that it has been shown to produce D-lactate [7,8]. The differences observed between these parasites are essentially quantitative. Energy yield in this group of cells is higher than in bloodstream-form *T. brucei*, with glucose consumption being between 5- and 10-fold lower when they are cultivated in the same conditions. Procyclic *T. brucei*, epimastigote and amastigote *T. cruzi*, promastigote and amastigote *Leishmania* and choanomastigote *Crithidia* all synthesize the majority of their ATP in the mitochondrion, which contains all of the enzymes of the Krebs cycle and a fully functional respiratory chain [9,10]. Moreover, in the insect stage, these parasites can use other energy sources such as amino acids (L-proline) [11].

GLUCOSE TRANSPORT IN EUKARYOTES AND PROKARYOTES

For any cell to use glucose, it must first obtain a supply of this sugar, either by its synthesis or via its acquisition from the environment through specific permeases. Studies on sugar transport in eukaryotes and prokaryotes have allowed the definition of three functionally distinct types of transport: (i) facilitated diffusion, (ii) secondary active transport coupled to the movement of ions and dependent on energy in the form of ATP, and (iii) direct active transport, where the substrate is phosphorylated while crossing the membrane by specific phosphotransferases involved in the uptake process. Only the first two types of transport are of relevance to kinetoplastids.

Facilitated diffusion

The transport of glucose via facilitated diffusion has been characterized in most mammalian tissues and a variety of other eukaryotic orders. A concentration gradient of sugar existing across the plasma membrane is required to drive this process (Figure 2a). The human erythrocyte transporter GLUT1 serves as model for this type of transport [12–14]. The inhibitors cytochalasin B, phloretin and to a lesser extent phloridzin have been shown to interfere with the facilitated diffusion of glucose.

Active transport coupled to the transport of ions

Sugar transport coupled to the transport of Na^+ has been

Figure 2 Hexose transport systems and inhibitors

The three different systems of hexose transport are represented. **(a)** Facilitated diffusion, of which the best characterized example is the mammalian erythrocyte glucose transporter GLUT1. **(b)** Glucose transport coupled to the transport of sodium, as in the glucose transporter found in the small intestine (SGLT1). **(c)** Hexose transport coupled to proton transport, for which the best characterized example is the lactose permease of *E. coli*. Abbreviation: Glc, D-glucose.

characterized in the mammalian intestine [15], where two plasma membrane proteins, the glucose/Na⁺ transporter and the Na⁺/K⁺-ATPase, act in concert to co-transport glucose down the energy-dependent Na⁺ gradient (Figure 2b). This process requires the hydrolysis of ATP, and is characterized by sensitivity to phloridzin [16,17], ouabain (inhibitor of the Na⁺/K⁺-ATPase) and monensin (Na⁺ ionophore).

Sugar transport coupled to H⁺ movement has been studied principally in prokaryotes, particularly *Escherichia coli*, with the lactose permease representing the classic example (Figure 2c) [18–20]. A H⁺-ATPase or the respiratory chain contribute to the

generation of the proton gradient. DCCD (*N,N'*-dicyclohexylcarbodi-imide), which is an inhibitor of the H⁺-ATPase [21], and the protonophore FCCP [carbonyl cyanide-4-(trifluoromethoxy)-phenylhydrazone] are typical inhibitors of proton-coupled transport systems.

GLUCOSE TRANSPORT IN KINETOPLASTIDS

African trypanosomes of the *T. brucei* group have been the most extensively studied kinetoplastids in terms of glucose transport, although similar studies have now been extended to representatives of most of the pathogenic species.

Glucose transport in the *T. brucei* group

Bloodstream forms

Southworth and Read [22,23] first demonstrated in *T. gambiense* the carrier-mediated uptake of glucose. *T. equiperdum* [24,25], *T. rhodesiense* [26] and *T. brucei* [27] have all been shown to possess carrier proteins for D-glucose. Radiolabelled glucose or non-metabolizable analogues have been used extensively to study uptake [27–29]. Glucose crosses the plasma membrane by facilitative diffusion. Affinity constants (K_m) of the transporters for D-glucose are summarized in Table 1. Significant variation is apparent, depending on the probe and experimental conditions employed by independent investigators. The purification and reconstitution in liposomes of the glucose transporter from *T. brucei* bloodstream forms confirmed that the transport is via facilitated diffusion [30].

One study, by Munoz-Antonia et al. [26], concluded that *T. rhodesiense* concentrates glucose via a Na⁺-dependent active transporter. However, these results have been questioned by Ter Kuile and Oppendoes [31] (Table 1).

At external glucose concentrations below 5 mM, the rate of the glycolytic flux does not exceed the rate at which glucose enters the cell [27]. Above 5 mM, however, the flux depends upon the rate at which hexokinase can phosphorylate the accumulated glucose [29]. Analysis of the substrate specificity of the bloodstream-form transporter [27,28] shows that hydroxy groups

Table 1 Kinetic parameters of glucose transport and type of transport in *T. brucei* forms

K_m values were determined by zero-trans uptake (*) or equilibrium-exchange (†) experiments. ND, not determined.

K_m (mM)		
<i>T. brucei</i> bloodstream forms	<i>T. brucei</i> procyclic form	References
0.90–1.18* (facilitated)	ND	[27]
0.90–1.54*	ND	[28]
ND	0.038–0.045* (active/H ⁺)	[36]
1.98† (facilitated)	ND	[29]
0.49† (facilitated)	ND	[33]
0.237*‡ (active/Na ⁺)	0.023*‡ (facilitated)	[26]
ND	2† (facilitated)	[31]
ND	0.052–0.059* (active/H ⁺ ; slippage)	[37]
ND	0.075–0.080*† (facilitated)	[40]
‡ <i>T. rhodesiense</i> .		

Table 2 Inhibition constants (K_i) for a range of D-glucose analogues

In some cases (*), K_m is given instead of K_i . For *Leishmania* and *Crithidia*, some values correspond to the percentage inhibition at the concentration given in parentheses. Values are from refs. ^a[28], ^b[37], ^c[36], ^d[44], ^e[43], ^f[56,57], ^g[58], ^h[103] and ⁱ[13].

Substrate/ analogue	Name	K_i (mM)					
		<i>T. brucei</i> bloodstream forms ^a	<i>T. brucei</i> procyclic forms ^b	<i>L. donovani</i> promastigote forms	<i>T. cruzi</i> epimastigote forms ^f	<i>Crithidia luciliae</i> ^g	GLUT1
Substrates of metabolism	D-Glucose	0.90	0.045 ^c –0.052*	0.019 ^d	0.059	0.11	4–10 ^h
	D-Mannose	0.67	0.031 ^c	~ 95% (10 mM) ^g	0.025	95% (10 mM)	14 ^h
	D-Fructose	2.56	1.5 ^c –2.54*	~ 90% (10 mM) ^g	0.338	97% (10 mM)	9300 ^h
	Glycerol	> 250	—	—	—	—	—
C-1	1-Deoxy-D-glucose	3.65	—	—	—	—	—
	1-Fluoro-1-deoxy-D-glucose	2.89	0.75	—	1.39	—	—
	Methyl α -D-glucoside	> 250	—	—	—	—	—
C-2	2-DOG	0.53	0.038 ^c –0.068*	0.024 ^d	0.248	0.22*	6.9 ⁱ
	2-Fluoro-2-deoxy-D-glucose	0.44	0.188	—	0.164	—	—
	D-Glucosamine	21.34	2 ^c –0.433	~ 70% (10 mM) ^g	1.32	—	—
	N-Acetyl-D-glucosamine	11.11	0.519	~ 80% (10 mM) ^g	0.156	—	—
	Mannitol	> 250	—	—	—	—	—
C-3	3-Deoxy-D-glucose	> 250	> 50	—	13.4	—	—
	3-Fluoro-3-deoxy-D-glucose	2.31	> 50	—	7.24	78% (10 mM)	—
	D-Allose	> 250	—	—	—	—	—
	3-O-Methyl-D-glucose	15.38	5.1 ^c	—	—	15% (10 mM)	20.9 ⁱ
C-4	D-Galactose	> 250	0.59 ^c –33	~ 5% (10 mM) ^g	61	50% (10 mM)	17–60 ^{h,i}
	4,6-Ethylidene-D-glucose	> 250	—	—	—	—	—
C-5	5-Thio-D-glucose	11.67	0.419	—	0.482	90% (10 mM)	—
C-6	6-Deoxy-D-glucose	1.54*	7.3	—	2.04	75% (10 mM)	—
	6-Chloro-6-deoxy-D-glucose	0.68	1.7	—	1.96	—	—
	6-Fluoro-6-deoxy-D-glucose	—	0.314	—	0.827	98% (10 mM)	—
	D-Xylose	> 250	—	—	—	—	60 ^h

located at carbon atoms C-3 and C-4 of D-glucose are vital for recognition by the transporter. The hydroxy groups at positions C-2 and C-6 are less important (Table 2). Eisenthal et al. [28] have proposed a model for the interaction between glucose and the transporter based on these studies (Figure 3a).

Glucose transport in *T. brucei* shows some important differences when compared with that by the human erythrocyte transporter GLUT1. For example, the parasite transporter is 1000-fold less sensitive to cytochalasin B [32,33] and, unlike GLUT1, the *T. brucei* transporter can transport D-fructose with a relatively high affinity (2.56 mM). Fry et al. [34] have shown that D-fructose is transported in the furanose ring form, in contrast with D-glucose which is transported in the pyranose form. In contrast with the mammalian erythrocyte glucose transporter, the *T. brucei* transporter will not recognize analogues of glucose altered at C-4 [28,35].

Procyclic forms

Studies performed on *T. brucei* procyclic forms (equivalent to the form found in the insect midgut) by Parsons and Nielsen [36] using low concentration of radiolabelled 2-deoxy-D-glucose (2-DOG) in zero-trans conditions showed the existence of a transporter with significantly higher affinity than that in bloodstream forms. Transport was sensitive to FCCP and KCN, suggesting possible proton-dependence. Ter Kuile and Opperdoes [31], however, using high concentrations of D-glucose in chemostat studies, concluded that *T. brucei* procyclics transport glucose via facilitative diffusion. In fact, the different experimental approaches used in these studies may underlie the discrepancy.

A recent study [37] showed that the inhibition of transport by

both FCCP and KCN is dependent on substrate concentration. These results suggest that transport may depend on a proton gradient at low substrate concentration, but not at higher concentrations. This process, termed 'slippage', has been described for other systems [38], and a mechanistic explanation has recently been given for the uncoupling of lactose transport from a proton gradient with increasing substrate concentration for the lactose permease of *E. coli* [39]. These data offered an explanation for the different observations in previous reports. Wille et al. [40] have also shown that glucose uptake by *T. brucei* procyclic forms occurs via a transporter with higher affinity than that in the bloodstream forms. At high external glucose concentrations, no effect on transport was elicited by FCCP, although studies at lower glucose concentrations were not performed. The procyclic transporter was also reconstituted in liposomes.

The procyclic-form transporter has an affinity for 2-DOG that is 10–20-fold higher than that of the transporter found in bloodstream forms [36,37,40] (Table 1). The specificity of the procyclic-form glucose transporter also differs from that of the bloodstream-form transporter, in that hydrogen bonds form with the hydroxy group at C-6 (Figure 3b; Table 2), which might explain the increased affinity for glucose shown by these cells. The procyclic transporter can also recognize and transport D-fructose with relatively high affinity (K_m 1.56 mM) [37].

Glucose transport in *Leishmania*

Glucose transport described in *Leishmania* has many features in common with that in procyclic *T. brucei*. Schaefer et al. [41] were the first to characterize uptake in *L. major* promastigotes, using 2-DOG as a substrate. Transport was suggested to be active, with

Figure 3 Proposed model of interactions between D-glucose and the hexose transporters of *T. brucei* bloodstream form (a), *T. brucei* procyclic form (b) and *T. cruzi* epimastigote form (c)

These models are based on those of Eisenthal et al. [28] for *T. brucei* bloodstream forms, Barrett et al. [37] for *T. brucei* procyclic forms, and Tetaud et al. [57] for *T. cruzi*. Hydrogen bonds between positions C-1, C-2, C-3, C-4, C-6 and the ring oxygen of glucose with the glucose transporter are indicated by the red zig-zag lines. Interactions between the less interactive hydroxy group of the C-2 carbon with the *T. cruzi* carrier are indicated by red dotted lines.

an apparent K_m of 0.16 mM. Uptake of labelled 2-DOG could be inhibited by substrate analogues with the following order of apparent affinity: D-glucose $\approx$ 2-DOG > D-fructose > D-mannose > N-acetyl-D-glucosamine > D-galactose [42,43] (Table 2). Moreover, inhibition by cytochalasin B and phloretin was relatively poor when compared with mammalian facilitative transporters.

Later, Zilberstein and Dwyer [44–46] characterized in *L. donovani* promastigotes (the equivalent of insect forms) a transporter with an apparent K_m of 24 μ M. The uptake system was proposed to involve a glucose transporter along with a H⁺-

Table 3 Kinetic parameters of glucose transport and type of transport in various kinetoplastids

Values for *Leishmania* were obtained with *L. donovani*, *L. enriettii* or *L. mexicana mexicana*. Values are from refs. ^a[27], ^b[28], ^c[29], ^d[33], ^e[26], ^f[36], ^g[37], ^h[40], ⁱ[31], ^j[44], ^k[49], ^l[50], ^m[48], ⁿ[56,57], ^o[58] and ^p[61].

Organism	Developmental stage	K_m (mM) for D-glucose or analogues	Type of transport
<i>Trypanosoma brucei</i>	Bloodstream trypomastigote	$\sim 1.0^{a,b,c,d,e}$	Facilitated
	Procyclic	$\sim 0.05^{e,f,g,h-2.0^i}$	Active H ⁺ or facilitated ^j
<i>Leishmania</i>	Promastigote	$\sim 0.02^{i,k,l}-0.6^m$	Active H ⁺ or facilitated ^m
	Amastigote	0.029 ^l	Active H ⁺ or facilitated
<i>Trypanosoma cruzi</i>	Trypomastigote	$\sim 0.150^n$	Facilitated
	Epimastigote	$\sim 0.08-0.3^n$	Facilitated
<i>Crithidia luciliae</i>	Choanomastigote	0.11–0.22 ⁿ	Facilitated
<i>Trypanosoma vivax</i>	Trypomastigote	0.585 ^p	Facilitated

ATPase which maintains the proton gradient that is critical for concentrative uptake [47].

Ter Kuile and Oppendoes [48], using a chemostat system, demonstrated that the intracellular concentration of glucose remained below the extracellular concentration with *L. donovani* promastigotes, and concluded that uptake was facilitated under these conditions (Table 3). Langford et al. [49] reported a transporter with a K_m for 2-DOG of 53 μ M in *L. enriettii* which was also sensitive to the protonophore FCCP.

Glucose transport was also investigated in amastigote forms of *L. mexicana mexicana* (amastigotes are the form that lives within macrophages of the mammalian host) [50]. These authors showed that amastigote forms of *L. mexicana* possess a glucose transport system, with an apparent K_m of 29 μ M, which is very similar to that identified in promastigote forms, although the two have different pH optima (promastigote, pH 7; amastigote, pH 5) (Table 3). Glucose uptake in amastigotes was also sensitive to different membrane potential/proton gradient antagonists (FCCP, DCCD, monensin), as previously characterized in *L. donovani* [44], although the question of direct proton-dependent transport remains an open one.

Glucose transport in *Trypanosoma cruzi*

The life cycle of *T. cruzi* is complex, with multiple developmental stages in both the reduviid insect vector and the mammalian host. Non-replicative bloodstream trypomastigotes and replicative intracellular amastigotes are found in the mammalian host, while epimastigote and metacyclic trypomastigote forms have been shown in the insect vector [51]. *T. cruzi* has a requirement for glucose or other biochemically interconvertible monosaccharides for growth, although it can survive for long periods in the absence of glucose. Culture-form epimastigotes have been shown to metabolize glucose [52], whereas bloodstream trypomastigotes appear to do so less intensively [53]; however, it seems that the different developmental stages of *T. cruzi* have qualitatively similar metabolic characteristics [54].

Warren and co-workers [55a,55b] reported that galactose supported respiration in epimastigote *T. cruzi*, and proposed the existence of an active galactose transporter at low concentrations

of this sugar and facilitative diffusion high concentrations. This may suggest a 'slippage' mechanism similar to that proposed for the procyclic-form glucose transporter in *T. brucei*.

Tetaud et al. [56,57] have described in *T. cruzi* a glucose transport system with many features in common with those in procyclic *T. brucei* and *Leishmania* promastigotes. A facilitated diffusion system with a high affinity for D-glucose (K_m 84.1 μ M) was identified in both epimastigote and trypomastigote forms (Table 3). The hydroxy groups at C-2, C-4 and C-6 are important in the interaction between the substrate (D-glucose) and the transporter. The hydroxy group at C-2 seems to be more important in recognition by the *T. cruzi* transporter compared with either of the *T. brucei* transporters, since the affinity for D-glucose is considerably higher than that for 2-DOG in the case of *T. cruzi* (K_i for D-glucose 0.059 mM; K_i for 2-DOG 0.248 mM) (Figure 3c; Table 2). The *T. cruzi* transporter can also transport D-fructose with relatively high affinity (K_m 0.682 mM) [57].

Glucose transport in epimastigote forms of *T. cruzi* is not sensitive to FCCP or KCN, regardless of substrate concentration (E. Tetaud, unpublished work), in contrast with that in *T. brucei* procyclics. Using ultra-pure D-galactose as a probe, we showed that this molecule is not a substrate for the *T. cruzi* transporter, in contrast with the observation of Warren and co-workers [55a,55b]. Strain-specific differences may account for this discrepancy, although the fact that the D-galactose is often contaminated with appreciable quantities of D-glucose could also explain it.

Glucose transport in *Crithidia luciliae*

Glucose transport described in the insect parasite *Crithidia luciliae* also shares many features with that in *T. brucei* procyclics, *Leishmania* and *T. cruzi*. Knodler et al. [58] demonstrated a high-affinity glucose transporter (K_m 0.22 mM) (Tables 2 and 3) which could also recognize D-fructose. Despite the fact that FCCP and phloridzin inhibited uptake, the authors failed to reveal the accumulation of substrate against a concentration gradient and concluded that the transporter is of the facilitated diffusion type.

Glucose transport in *Trypanosoma vivax*

Metabolism of glucose by bloodstream forms of *T. vivax* is similar to that in the intracellular parasites *T. cruzi* and *Leishmania* [59], although this organism dwells free in the mammalian bloodstream (as does *T. brucei*) [60] and possesses a partial Krebs cycle.

Waitumbi et al. [61] have shown that *T. vivax* bloodstream forms contain a saturable glucose transporter with a typical facilitated-carrier mechanism. The affinity for D-glucose is similar to that of the *T. brucei* bloodstream-form carrier (K_m 0.548 mM) (Tables 2 and 3), suggesting that the evolution of these lower-affinity transporters is an adaptation to living in the glucose-rich environment of the mammalian bloodstream. Cytochalasin B and phloretin inhibited glucose uptake in *T. vivax*, although no inhibitors of ion-dependent transport (monensin, ouabain, FCCP and phloridzin) had an effect when assaying in conditions of high external glucose concentration. D-Fructose is also a competitive substrate of glucose uptake in *T. vivax*.

Summary

The kinetoplastid glucose transporters all share the ability to carry D-fructose, which distinguishes them from the mammalian GLUT1 erythrocyte transporter. Other mammalian hexose transporters (GLUT2 and GLUT5) [62,63] do transport D-fructose, but with significantly lower affinities than these kinetoplastid

ones. Relatively low sensitivity to phloretin and cytochalasin B also distinguish the kinetoplastid hexose transporters.

The affinity of the transporter for its substrate correlates with the glucose concentration in the environment in which the parasites find themselves. Thus *T. brucei* and *T. vivax* bloodstream forms have low-affinity, high-capacity transporters, whereas parasites dwelling in the insect, or intracellularly where free glucose is not abundant (being metabolized rapidly by the host cell), have higher-affinity transporters.

Some of the kinetoplastid cell types have been shown to transport glucose via facilitative diffusion, while in other cases controversy has surrounded the issue of whether transport is proton-coupled or not. To date, definitive experiments to resolve this controversy have not been performed. Undoubtedly, transport in procyclic *T. brucei* and *Leishmania* species can be inhibited by classical inhibitors of proton-dependent transport, and this inhibition has been shown to depend on substrate concentration in the case of *T. brucei*. However, these pharmacological reagents can also inhibit other phenomena, including plasma membrane potential, and further studies are required to distinguish between true secondary active transport involving protons and other events leading to decreased activity of the permeases.

STRUCTURE OF GLUCOSE TRANSPORTERS IN EUKARYOTES AND PROKARYOTES

Model structure of the human erythrocyte facilitated glucose transporter GLUT1

In 1985, Mueckler et al. [64] cloned and sequenced a gene encoding the human erythrocyte facilitated glucose transporter GLUT1. Analysis of the sequence using a hydrophobicity profile program [65] led to a proposed model for the secondary structure in which the transporter was composed of 12 putative hydrophobic transmembrane segments separated by hydrophilic loops. N and C-termini were located on the cytoplasmic side (Figure 4). Two large hydrophilic loops, one external between the putative transmembrane domains 1 and 2, containing an N-glycosylation site, and the other internal between transmembrane helices 6 and 7, were found. This model was supported by other workers, using proteolytic digestion of the GLUT1 transporter in conjunction with antibodies directed against different segments of the protein [12,14,66]. More support came from Hresko et al. [67], who expressed GLUT1 mutants containing glycosylation sites in each hydrophilic segment in *Xenopus* oocytes, to study their orientation in the membrane. The model is now generally accepted.

Facilitated and active transporters

Two major classes of glucose transporter have been identified in eukaryotes: (i) those related in structure and sequence to the mammalian facilitated glucose transporter GLUT1; and (ii) the Na⁺-dependent glucose transporters, e.g. the mammalian intestinal brush-border and kidney glucose transporter SGLT1. The first family comprises many examples from organisms as diverse as bacteria, yeast, plants and humans [12]. Members of this family share significant amino acid sequence identity and are believed to possess similar membrane topologies. In contrast, the Na⁺-dependent transporters are not related in sequence to the first family, and are believed to have a different topology [15]. Several proteins belonging to the GLUT1 superfamily, e.g. the *E. coli* arabinose and xylose transporters [68–76] and some eukaryotic hexose transporters [77,78], were also shown to be active proton symporters.

Figure 4 Schematic representation of the proposed topology of the GLUT1 transporter in the membrane

The representation is based on the model of Mueckler et al. [64]. The numbered pink boxes indicate hydrophobic segments which form the 12 transmembrane domains, and the solid red circle indicates the N-linked glycosylation site.

KINETOPLASTID HEXOSE TRANSPORTERS

Hexose transporter family from *Leishmania* spp.

In 1989, Cairns et al. [79] cloned from *Leishmania enriettii* a cDNA encoding a protein with characteristics typical of a hexose transporter. Subsequently this cDNA (Pro-1) was used to identify a multigenic family with at least eight clustered members (Figure 5a). Detailed analysis of the 3' repeat units revealed the presence of two isoforms (Iso-1 and Iso-2) [80] which differed in their N-termini, with Iso-1 containing a unique hydrophilic extension (Figure 6) [81]. Northern blot analysis revealed expression to be principally in the insect form (promastigote; hence Pro-1). Pro-1 possesses a number of peculiarities when compared with the mammalian GLUT1 transporter. Notably, a number of cysteine

residues are present in the first extracellular loop, and the loop between the transmembrane domains 6 and 7 is relatively small. No potential N-linked glycosylation site was identified in the first extracellular loop. Piper et al. [82] have shown, using antibodies directed to Iso-1 and Iso-2, that they differ in subcellular localization, the former being found principally in the flagellar membrane and the latter in the plasma membrane and flagellar pocket. The different N-terminal sequences may target the different isoforms.

Iso-1 and Iso-2 have been functionally expressed in *Xenopus* oocytes, where both can transport 2-DOG [49] with substrate-inhibition and pharmacological profiles similar to those of the glucose transporter identified in promastigotes. Kinetic parameters measured in oocytes are not, however, comparable with

Figure 5 Genomic organization of the hexose transporters from *Leishmania enriettii* and *Trypanosoma brucei*

(a) *L. enriettii*. Black boxes indicate the conserved coding sequences of the *Pro-1* genes, and the preceding small boxes indicate the unique N-terminal sequences of either Iso-1 (white box) or Iso-2 (pink boxes). The entire repeat contains at least eight copies. (b) *T. brucei*. Black and pink boxes indicate the coding regions of the *THT1* and *THT2* genes respectively. A hybrid gene (*THT1e*), corresponding to *THT1* with a *THT2* 3' terminus, is depicted by a composite black and pink box.

Iso-1	MSYPPPKSQD	QGQLPLKTF	SPPRRTGTS	HAHNDCVAE	SETLPTTPPL	PSFLRGNDVQ	LPKTSVAHS	LTTSPSPVNN	LSPGAGPHTH	HRIANPINPA	STEDDTTFTT	TSASQDPPRE	SSLFSSLINR	130		
Iso-2									MSDRVEVN.E	RRSDSVSEKE	PARDDARKDV	TDQDQAP	PMFTANNAR	46		
THT1									..MTERRDNV	SHAPDAIEGP	NDGAHAEDTS	PGFFSLENLG	38			
THT2									..MTERRDNV	SHAPDAIEGP	NDGAHAEDTS	PGFFSLENLG	38			
TcrHT1													29			
TvHT1													38			
D2			..MGSPNDKEE	EGTREPFHSL	EVRGQVPAA	ALTMISTNM	SPKEQLSNGK	SITQANADLP	LISAADSPS	MTLKKRSSAP	ETLSLEDDE	EEDSPQPLSN	TPFFSMKNLI	109		
Iso-1	AILVQAIGGS	LNQYSIGFVG	VYSTLFGYST	NCASFQENS	CTTVPNADCK	WFSVPTGSSY	CGWPEVTCRK	EYAYSSPAEM	PGALARCEAD	SRCRWSYSDE	EQCNPSGYSS	SESGIFAGSM	IAGCLIGSVF	260		
Iso-2	VMLVQAIGGS	LNQYSIGFVG	VYSTLFGYST	NCASFQENS	CTTVPNADCK	WFSVPTGSSY	CGWPEVTCRK	EYAYSSPAEM	PGALARCEAD	SRCRWSYSDE	EQCNPSGYSS	SESGIFAGSM	IAGCLIGSVF	176		
THT1	VAQVQVGGT	LNQYVIGYVA	VYLLLYLAT	ECK.FTTEGA	CGAKIYGCK	WSGTT								138		
THT2	VAQVQVGGT	LNQFSIGFVA	VYLLLYLAT	NCSLFKTTEA	CKAVGSGCE	WKDTEV								139		
TcrHT1	VAQVQVGGT	LNQFSIGFVA	VYAYFYLMST	DCSMYKKEVA	CNRVLNABCS	WNKTRGE								148		
TvHT1	IQVQVSTGS	LNQFSIGFVA	VYMLHYEIFS	GCSALESSGA	CSG..NSKCT	WIPNNST								136		
D2	VATPIILTF	LYGNLGFVG	PYSTMYGYAS	NCQLYSAKS	CETLTAACKR	WPNASTVSN	TTYGVEGCV	ADR.TTCFLK	YSDEAGCLSD	SACKWYSYAN	TCGNQVGYSS	IQSGVFAAGL	VIGSTMGALM	237		
Iso-1	AGPLASKIGA	RLSFLVLGLV	GVAASVMY	HA.SCAADEFW	VL	IVGRFVIGLF	LGVICVACPV	YTDQNAHPKW	KRTIGVGMFQV	FTTLGIFVAA	LMGLALGQSI	RFDDHGDQKV	MARMQGLCVF	STLFSLLTVV	390	
Iso-2	AGPLASKIGA	RLSFLVLGLV	GVAASVMY	HA.SCAADEFW	VL	IVGRFVIGLF	LGVICVACPV	YTDQNAHPKW	KRTIGVGMFQV	FTTLGIFVAA	LMGLALGQSI	RFDDHGDQKV	MARMQGLCVF	STLFSLLTVV	306	
THT1	AGKCIITFFGL	KKSFIIVSIT	CTIACVQV	QV.AIEYNNY	AL	CTGRVLIGLG	VGILCSVCPM	YVNEAHPKL	CKMDGVLFQV	FTTLGIMLAA	MLGLILDKTV	ASKEEAN	..M.AGR	LHVSFAV	PLGLSVAMFL	266
THT2	AGKCIITFFGL	KKSFIIVSIT	CTIACVQV	QV.AIEYNNY	AL	CTGRVLIGLG	VGILCSVCPM	YVNEAHPKL	CKMDGVLFQV	FTTLGIMLAA	MLGLILDKTV	NYDNDP	..M.AGR	PHGFCFV	SSVLVSAMFL	267
TcrHT1	AGQFAARFGL	KVSFLVIGIV	GVSVMY	HV.SSATNEFW	VL	CVGRLLIGLV	LGVLNVACPM	YVDQNAHPKL	LHVDGVLFQV	FTTFGIMFPA	AMGLAIGQSV	NFDKIDK	..M.DAR	MOQYCAF	STLLSVLMVA	276
TvHT1	AGKFLSKFLG	KMSFIIVSGV	GIVGSALI	HV.ATRGSTLW	VM	CVGRFLMGLV	LGVLNVACPM	YVNEAHPKY	RKTIGVLFQV	FTTFGIMFPA	LLGLAIVKTP	GHDKASG	..L.LWR	MOVFCV	STALLSALLV	264
D2	GYLTKRLDY	CKSLFPIGLL	SVIGNVLT	HV.ATGLFHYW	VL	FVARIVLGF	LGWQSIITSH	YTDKFAFANH	AKTLTGILFQV	SVSTGIFVTS	FFGLVLGNTI	QYDAASNANT	MGRMQGLVSV	STLLSIFVFP	367	
Iso-1	LGIIVTRESRA	KFD...GGE	EGRAE...LNP	SEYGYVEMIP	RLLMGCVMA	TQLQTGINAV	MNYAPTIMG	LGALPLVGNF	VVLMNFFVT	LASIPLSYVF	TMRHVFLPFS	IFTFCMLFM	CGIPVYPGVS	514		
Iso-2	LGIIVTRESRA	KFD...GGE	EGRAE...LNP	SEYGYVEMIP	RLLMGCVMA	TQLQTGINAV	MNYAPTIMG	LGALPLVGNF	VVLMNFFVT	LASIPLSYVF	TMRHVFLPFS	IFTFCMLFM	CGIPVYPGVS	430		
THT1	VGMLFRESTA	TFA...QDD	DGKADGMDP	NEYGWQMLM	PLFMGAVTAG	TQLQTGINAV	MNYAPTIMG	LGMDPSLGNF	LVMAWNFVTS	LVAIPLASRF	TWRQMFTICS	FVASCMLCFL	CGIPVFPVGA	392		
THT2	VGMLFRESTA	TFS...QDD	DGKADGMDP	NEYGWQMLM	PLFMGAVTAG	TQLQTGINAV	MNYAPTIMG	LGMDPSLGNF	LVMAWNFVTS	LVAIPLASRF	TWRQMFTICS	FVASCMLCFL	CGIPVFPVGA	393		
TcrHT1	LGIIFLGESKT	KFT...SGK	HDDGTALDP	NEYSYLQMLG	PLAMGLVTS	TQLQTGINAV	MNYAPKIMG	LGMPVLVGNF	VVMAWNFVTS	LVSIPLARVL	TWRQLFLGAS	FVASCMLCFL	CGIPVFPVGA	402		
TvHT1	LGLVVRKST	SFA...GGV	DSAGEBVLDP	NEYSVRQMLG	PLAMGLVTS	TQLQTGINAV	MNYAPEIMRN	IGMDPMENGS	AVMSWNFVTS	LVAIPLASRF	TWRQLFLACS	FVASCMLCFL	CGIPVFPVGA	390		
D2	LPLITKDGYS	KSRRGDYE	NSDASRKAA	BEYTMQIMIG	PILNGVAMGC	TQLQTGINAN	MNPATTIMN	LGLOPLVGNF	IVMAWNMLAT	PCVILPSRRF	SMRLTLFPCG	FVGLSCLCVFL	GGIPVFPVGT	497		
Iso-1	KKLEAKNGVA	ITGILLFILG	FEVCGVPCY	VLTQDMFP	PPS	FRPRGASFTQ	VAQFIFNLII	NVCYPIATES	ISGGPSGNQD	KQQAFAFIFF	GGLGLICFVI	QVFFLHPWDE	ERDGGKVVAP	AIGKKELSEE	644	
Iso-2	KKLEAKNGVA	ITGILLFILG	FEVCGVPCY	VLTQDMFP	PPS	FRPRGASFTQ	VAQFIFNLII	NVCYPIATES	ISGGPSGNQD	KQQAFAFIFF	GGLGLICFVI	QVFFLHPWDE	ERDGGKVVAP	AIGKKELSEE	568	
THT1	GK.EVKNGVA	TTGIALFIAA	FEFGVSGCF	VLAQDLFPPS	FRPKGSSFV	MMQFIFNLII	NLLYPITTEA	ISGGATGNQD	KQQAFAFIFF	GGLGLICFVI	QVFFLYPYDA	N.....	QDHNED	508		
THT2	EE.KVNGVA	TTGIALFIAA	FEFGVSGCF	VLAQDLFPPS	FRPKGSSFV	MMQFIFNLII	NLLYPITTEA	ISGGATGNQD	KQQAFAFIFF	GGLGLICFVI	QVFFLYPYDA	N.....	QDHNED	509		
TcrHT1	DK.NVNGVA	TTGIAVFIAA	FEIPLGSCFF	VLAQDLFPRS	FRPKGSSFV	LTMFIFNVII	NVCYPIATES	ISGGPSGNQD	KQQAFAFIFF	GCIGLVCFVL	QVFFLYPYDE	S.....	TPQNDHT	520		
TvHT1	SV.DNRNIVA	TTGIAVFIAA	FEIPLGSCFF	VLAQDLFPRS	FRPKGSSFV	VAQFIFNLII	NLLYPITTEA	ISGGKSGPSE	KQQSVSFIIF	GIGIICFVL	QVRYLTPWED	GQGTSTSPTA	RCNAPTSPNN	519		
D2	KSDKAISGLIA	ITGIAIFLIA	YEMGVGCFY	VLAQDLFPPS	FRPKGSSFV	GVMFIFNLII	NICYPIATBG	ISGGPSGNPN	KQQAFAFIFF	GCIGVAVCVI	EYFFLPQWVE	PEAKMTDLDL	GAAPVEGKHD	627		
Iso-1	SIGNRAE...													651		
Iso-2	SIGNRAE...													567		
THT1	HGGEFVPEQKT	YPVE	ASPRN											527		
THT2	HGTEFVERIA	SPVDVPTPRN												529		
TcrHT1	NEESALPERQ	SPIEVATPGN	RQAA											544		
TvHT1	GEGETATADM	SPVEMSTPKH	SGAA											543		
D2																

Figure 6 Alignment of the predicted amino acid sequences of kinetoplastid glucose transporters

Identical amino acids are shown by stars, and gaps are indicated by dots. The 12 putative hydrophobic segments are overlined and numbered 1–12. The cysteine residues located in the first loop are indicated by arrows. Underlined sequences refer to potential N-linked glycosylation sites. The four arginine residues conserved between the mammalian hexose transporters and the kinetoplastid transporters are highlighted in pink. The shaded boxed region is the sequence that is conserved between transporters expressed in the insect-form parasites. The alignment was performed with the Pileup program from the GCG Wisconsin Sequence Analysis Software Package, using the default parameters.

those measured in promastigotes (Table 4). Significant differences in K_m values between the two isoforms were not found, suggesting they both encode transporters with similar specificity, differing only in their localization. The discrepancy in K_m measurements between promastigotes and transporters expressed in oocytes might imply that the major promastigote transporter has yet to be cloned, or that technical limitations in measuring the K_m of the transporters in the oocytes or in live parasites need to be overcome.

Langford et al. [83] also cloned from *L. donovani* two further genes with identity to the glucose transporter superfamily, which they called D1 and D2. Both genes are present as single copies. D2 is very similar to Pro-1 and possesses a long N-terminal extension like Iso-1. Expressed in *Xenopus* oocytes, a K_m for 2-DOG of ~150 mM (Table 4) was measured, and D-fructose and D-mannose inhibited transport. The D2 gene is expressed chiefly in insect-stage parasites, and the transporter is located primarily in the plasma membrane. It is possible that D2 encodes a transporter adapted to function directly after an insect meal, when the concentration of sugar reaches very high levels and so a high K_m would be advantageous [43]. A cysteine-rich first exofacial loop is conserved in D2 (Figure 6).

In contrast, D1 is structurally quite different from either D2 or Pro-1, and its expression is not regulated during the parasite life

cycle. D1 is more similar in sequence to the mammalian transporter GLUT1, particularly in the conservation of a relatively large loop separating transmembrane segments 6 and 7. The functional expression of the D1 gene in *Xenopus* oocytes revealed it to encode a plasma membrane myo-inositol/H⁺ symporter rather than a hexose transporter [84].

Hexose transporters of *Trypanosoma brucei*

In 1992, Bringaud and Baltz [85] cloned a gene from *T. brucei* with great similarity to the *Leishmania enriettii* Pro-1 gene. This gene, called THT1 (for Trypanosome Hexose Transporter), was used to identify a multigenic family consisting of two isoforms (THT1 and THT2) [86] (Figure 6) encoding proteins that are 80% identical, with the differences located principally in the first extracellular loop and the C-terminus (Figure 6). This first loop shares with the *Leishmania* hexose transporters a conserved array of cysteine residues (Figure 6). THT2, but not THT1, has a putative N-linked glycosylation site in this exofacial loop, and both isoforms have a second potential glycosylation site in the N-terminus.

In *T. brucei* strain EATRO 164, these genes are arranged in a tandem repeat located exclusively to one of the megabased sized

Table 4 Kinetoplastid hexose transporters: kinetic parameters and recognition pattern

The level of inhibition is indicated as follows: —, inhibition of 5–20%; $\pm$, 20–40%; ++, 40–70%; + + +, greater than 70%. The various forms investigated were: *, promastigote forms; †, bloodstream forms; ‡, procyclic forms; §, epimastigote forms.

	Glucose transport in parasite		Expression of glucose transporter genes in <i>Xenopus</i> oocytes or CHO cells			Inhibition of glucose uptake by C-3 and C-6 analogues			
	K_m for D-glucose or 2-DOG (mM)	Type of transport	K_m for 2-DOG	Type of transport	Transport of D-fructose/ K_m (mM)	In the parasite		In CHO cells	
						Position C-3	Position C-6	Position C-3	Position C-6
<i>Leishmania</i> * (Iso-1)	0.053	Active/facilitated	0.647 ± 0.26	Active/facilitated	+ /ND	ND	ND	ND	ND
<i>Leishmania</i> * (Iso-2)	0.053	Active/facilitated	0.285 ± 0.06	Active/facilitated	+ /ND	ND	ND	ND	ND
<i>Leishmania</i> * (D2)	0.024 or 0.6	Active/facilitated	~ 150	Active/facilitated	+ /ND	ND	ND	ND	ND
<i>T. brucei</i> † (THT1)	0.5–2.0	Facilitated	ND	Facilitated	+ /2.56	++	+++	ND	ND
<i>T. brucei</i> ‡ (THT2)	0.038–0.08 or 2.0	Active/facilitated	0.151 ± 0.01	Facilitated	+ /2.54	—	—	—	$\pm$
<i>T. cruzi</i> § (TcrHT1)	0.08–0.3	Facilitated	0.315 ± 0.01	Facilitated	+ /0.682	—	—	—	$\pm$
<i>T. vivax</i> † (TvHT1)	0.585	Facilitated	0.545 ± 0.02	Facilitated	+ /ND	ND	ND	—	++
GLUT1	4–10	Facilitated	~ 7	Facilitated	— /9300	++	ND	ND	ND

chromosomes, with six copies of *THT1* and five copies of *THT2* (Figure 5b) [87,88]. Analysis of polymorphism in gene copy number for both isoforms in numerous strains revealed them to be present in multiple copies in tandem arrays, with copy number varying in a strain-specific manner. Two-thirds of the strains studied were heterozygous for *THT1* gene copy number. The clusters were located on two homologous chromosomes, as judged by analysis of progeny from crosses between clones. Following a detailed DNA sequence analysis, it was proposed that *THT1* and *THT2* evolved from a common ancestor by duplication and subsequent modification (insertion of a DNA fragment and point mutations) [87]. Northern blot analysis revealed that expression of these genes is life-cycle-stage-dependent. Bloodstream forms express 40-fold more *THT1* than *THT2*, whereas procyclics express *THT2* but no detectable [86] (or very low levels of) *THT1* mRNA, depending on the strain (F. Bringaud and T. Baltz, unpublished work). An observation that *THT2* expression could be regulated by levels of external glucose [86] was difficult to reproduce [88,89], and expression of this isoform appears to be constitutive. The 3' untranslated region of *THT1* confers stability on this transcript in the bloodstream form and instability in procyclic forms [89].

THT1 and *THT2* have both been expressed in different systems to verify their function. *Xenopus* oocytes microinjected with *THT1* mRNA showed an increase of 2-DOG uptake, and the substrate and pharmacological specificity of the expressed transporter correlated with that of glucose uptake in bloodstream-form *T. brucei*. It was concluded that the *THT1* gene encodes the facilitated transporter identified in bloodstream-form *T. brucei*.

The expression of *THT2* has been performed in two systems [37]: (1) *Xenopus* oocytes, where the function of the hexose transporter was confirmed and substrate specificity determined, although kinetic parameters were not measured; and (2) CHO cells, where difficulties frequently found in deriving kinetic parameters from the oocyte system [49] were circumvented. In this latter system a K_m for 2-DOG of $151 \mu\text{M}$ was reported, which is of the same order as the K_m value measured in procyclic cells ($K_m \sim 80 \mu\text{M}$) (Table 4). The *THT2* transporter expressed in CHO cells shared a profile of substrate recognition with the transporter in procyclic trypanosomes (Table 3). In contrast with 2-DOG transport in procyclic cells, FCCP and KCN had no effect on transport of 2-DOG in CHO cells expressing *THT2* (see section on glucose transport in procyclic *T. brucei*). The presence of auxiliary factors in the parasite membrane may influence the

mode of transport [90], as may changes in the conformation of the transporter in the heterologous system. The effects of FCCP and KCN could occur at a level involving events other than transport itself. However, it was clear that, in the CHO cell system, the *THT2* permease acted as a facilitated transporter, in contrast with the procyclic cells, where an influence was exerted by protons at low substrate concentrations.

Hexose transporter of *Trypanosoma cruzi*

Using the *T. brucei* glucose transporter gene (*THT1*) as a probe, Tetaud et al. [56] identified a clone containing a 15 kb insert from *T. cruzi* strain C.L. Analysis of this clone revealed the presence of an open reading frame, which was called *TcrHT1* (for *T. cruzi* Hexose Transporter), encoding a protein with the characteristics of a hexose transporter (see above) (Figure 6). As with the other kinetoplastid hexose transporters, *TcrHT1* possesses a highly conserved array of cysteine residues in the first exofacial loop. A potential N-linked glycosylation site was also noted in this loop. At least eight copies of the *TcrHT* gene are tandemly reiterated on one of the chromosomes of *T. cruzi* C.L.

No second related transporter gene could be found, suggesting that *T. cruzi* possesses only a single hexose transporter isoform. Northern blot analysis of trypomastigote and epimastigote total RNA revealed two mRNA transcripts of equal abundance. Analysis of the cDNA corresponding to these two transcripts revealed that the 3' untranslated regions differed in sequence and size, with one corresponding to the sequenced genomic clone and the other shorter. Analysis of intergenic regions between *TcrHT1* copies revealed a corresponding sequence and size difference, thus explaining the presence of two transcripts.

The *TcrHT1* gene has been expressed in *Xenopus* oocytes [56] and CHO cells [57]. In both systems the protein transported 2-DOG with a qualitatively similar substrate specificity and pharmacology as the transporter identified in epimastigotes of *T. cruzi*. In the CHO system, the K_m of the *TcrHT1* permease for 2-DOG (0.314 mM) was almost identical to that measured in epimastigotes (0.312 mM) (Table 4). The *TcrHT1* transporter can also transport D-fructose.

Having established the features of the substrate that are critical to catalytic activity (Figure 3), we tried to determine amino acid residues that may be important in substrate recognition, by chemical modification of the transporter in both *T.*

Figure 7 Phylogenetic tree of the kinetoplastid hexose transporters

The length of each pair of branches represents the distance between sequence pairs. The scale beneath the tree measures the distance between sequences. Units indicate the number of substitution events. The phylogenetic tree was generated using MEGALIGN of the Lasergene System (DNASTAR Inc.), using the default parameters.

cruzi and CHO cells expressing the *TcrHT1* gene. We suggested a role for arginine in substrate recognition, as arginine-modifying reagents inactivated the transporter in a manner that was protected by D-glucose [57].

Hexose transporter of *Trypanosoma vivax*

Waitumbi et al. [61] used degenerate oligonucleotides corresponding to conserved amino acid sequences found in other trypanosomatid hexose transporters to identify a gene encoding a homologous protein in *T. vivax* (see above) which was called TvHT1 (for *T. vivax* Hexose Transporter) (Figure 6). The first extracellular loop was once again found to possess numerous conserved cysteine residues (Figure 6); it was also shown to possess two potential N-linked glycosylation sites. The *TvHT1* gene is present in multiple copies, organized as a tandem repeat of at least six copies.

Northern blot analysis of RNA from bloodstream-form *T. vivax* revealed two different transcripts which have arisen from the use of alternative polyadenylation sites [61]. Unlike *T. brucei* and *Leishmania*, but like *T. cruzi*, it was suggested that *T. vivax* possesses only one hexose transporter isoform.

Functional expression of the *TvHT1* gene in CHO cells confirmed the function of the gene and revealed a substrate specificity intermediate between those of bloodstream-form and procyclic *T. brucei* (Table 4). In the CHO cell system, a K_m for TvHT1 of 0.548 mM was determined, which is close to that detected in bloodstream-form parasites (0.585 mM). The permease behaves as a facilitated transporter when challenged pharmacologically, and also uses D-fructose as a substrate.

Comparative analysis of amino acid sequences of the kinetoplastid hexose transporters

Alignment of the amino acid sequences of the different hexose transporters from *T. brucei* (THT1 and THT2), *Leishmania* (Iso-1, Iso-2 and D2), *T. cruzi* (TcrHT1) and *T. vivax* (TvHT1) revealed great similarity between all of these proteins (30–85% similarity, with most conservation in the central part and towards the C-terminus of the protein) (Figure 6). The *L. donovani* D2 transporter was the most divergent (Figure 7), suggesting a distinct physiological role [43] consistent with its extremely high K_m for D-glucose. The extreme N- and C-termini as well as the

first extracellular loop vary a great deal between the transporters. Other hexose transporters from mammals, yeast and bacteria also diverge most in their N- and C-terminal sequences [12]. In contrast with the mammalian hexose transporters (GLUT), N-linked glycosylation sites are not always found in the first extracellular loop (Figure 6), suggesting that the kinetoplastid transporters do not necessarily require glycosylation for activity.

In all the kinetoplastid hexose transporters, the first extracellular loop is characterized by a highly conserved arrangement of cysteine residues, even though the remaining sequence of the loop diverges significantly between species. Cysteine-rich proteins are often associated with membranes in eukaryotes [91–97] and, intriguingly, many kinetoplastid surface proteins, including the variant surface glycoproteins of *T. brucei*, share the cysteine-rich motif observed in the hexose transporter. This motif may therefore play a crucial role in the membrane architecture of these parasites, or may be critical for the folding of extracellular domains of membrane proteins in order to prevent cleavage by the numerous proteases present in the blood and intestinal tract of hosts [92,95,96,98,99].

Analysis of the region between the third and fourth transmembrane segments revealed a high level of similarity between the sequences of *T. cruzi* TcrHT1, *Leishmania* Pro-1, Iso-1 and Iso-2 and *T. brucei* THT2 (Figure 6). All of these transporters are expressed principally in forms of the parasites that live in the insect midgut. All also possess relatively high affinity for D-glucose, in contrast with THT1 and TvHT1, which are expressed in bloodstream-form parasites and have lower affinity for glucose. This region may therefore contribute to the increased affinity for glucose shown by transporters responsible for glucose uptake in insects; however, direct evidence does not yet exist to support this, and it is the extreme C-terminus that appears to play a critical role in substrate affinity in the mammalian GLUT transporters [100].

The secondary structure of all members of the glucose transporter superfamily is conserved, bearing 12 putative transmembrane hydrophobic domains. Residues present in helix 7 have also been implicated in the recognition of substrate [13]. Recent studies using chimaeras of GLUT2 and GLUT3 showed that substrate specificity (GLUT2, but not GLUT3, also transports fructose) is determined by helix 7, as is a large component of the binding affinity [101]. Inspection of the aligned sequences of the trypanosomatid transporters reveals great sequence similarity in this region (Figure 6), although the *T. cruzi* transporter has a striking difference in that Ser-321 replaces an otherwise conserved alanine (or glycine) residue. It was proposed that this amino acid change may underlie the difference in affinity observed between 2-DOG and D-glucose in *T. cruzi* which is not observed in the other species. Analysis of TcrHT1 mutated at this amino acid (Ser → Ala) in CHO cells failed to support this hypothesis, although technical limitations leave the question open (E. Tetaud, unpublished work).

Tetaud et al. [57] used amino acid modifying reagents to identify potential amino acids involved in the interaction with substrate. Arginine was shown to play such a role. Comparison of the different sequences of known kinetoplastid hexose transporters (Pro-1, D2, THT1 and THT2, TcrHT1 and TvHT1) revealed four conserved arginine residues. The residues are located in transmembrane helix 4 and between transmembrane helices 5/6, 8/9 and 10/11. Three of these residues (those located in transmembrane helix 4 and between transmembrane helices 8/9 and 10/11) are highly conserved in other members of the glucose transporter superfamily [12], and one or more of these may be critical in substrate binding. Recently Wandel et al. [102] demonstrated that the arginine residue located in the loop

between transmembrane domains 8/9 of the mammalian glucose transporter GLUT4 plays a direct role in glucose uptake.

CONCLUSIONS

Genes for seven hexose transporters and a homologous *myo*-inositol transporter have been cloned from parasitic protozoa belonging to the order kinetoplastida. The genes have been expressed in heterologous systems (either *Xenopus* oocytes or CHO cells) to verify function, and generally it has been possible to identify the genes as those encoding transporters expressed at different stages of the parasite life cycle. Differences exist in substrate affinity between the different transporters in a manner that may be related to the physiological conditions experienced by these parasites. For the *Leishmania* transporters, differential subcellular localization has been noted using antibodies to detect products of the different genes, although the physiological basis for such targeting remains to be elucidated. These transporters are structurally similar to other members of the glucose transporter superfamily, and can be expressed in heterologous systems. Given the wide variety of functionally distinguishing features (substrate specificity and kinetic parameters), the kinetoplastid hexose transporters offer an exceptional range of material to assist in elucidating the mechanism of this class of membrane transporter in general. Moreover, the specific elucidation of functional properties of the parasite transporters may allow novel approaches to therapy against these diseases. As surface molecules, the transporters may be immunogenic and exposed to the immune system. Particularly in the case of bloodstream-form African trypanosomes, glucose represents the sole energy source for these organisms, and thus inhibitors of the transporter would be expected to kill the parasites. Moreover, given the high capacity of these transporters, they may represent a means of targeting novel toxins to the cells, if these toxins can be coupled to chemical motifs specifically recognized by the kinetoplastid hexose transporters.

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