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Characterization of glucose transport and cloning of a hexose transporter gene in *Trypanosoma cruzi*

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ABSTRACT **A gene from *Trypanosoma cruzi*, TcrHT1, which encodes a member of the glucose transporter superfamily has been cloned. The gene is similar in sequence to the *T. brucei* hexose transporter THT1 and the *Leishmania* transporter Pro-1 and is present in the *T. cruzi* genome as a cluster of at least eight tandemly reiterated copies. Northern blot analysis revealed two mRNA transcripts which differ in size with respect to their 3′ untranslated regions. When injected with *in vitro* transcribed TcrHT1 mRNA, *Xenopus* oocytes express a hexose transporter with properties similar to those of *T. cruzi*. Glucose transport in *T. cruzi* is mediated via a carrier with unique properties when compared with the other glucose transporters already characterized among the Kinetoplastida. It is a facilitated transporter with a high affinity for D-glucose (K_m = 84.1 ± 7.9 μM and V_{max} = 46 ± 9.4 nmol/min per mg of protein) that shares with other kinetoplastid hexose transporters the ability to recognize D-fructose, which distinguishes these carriers from the human erythrocyte glucose transporter GLUT1.**

The protozoan parasite *Trypanosoma cruzi* causes Chagas disease in Central and South America. The life cycle of *T. cruzi* involves multiple developmental stages in both the reduviid insect vector and the mammalian host. *T. cruzi* is found transiently as a nonreplicative bloodstream trypomastigote which invades cells and differentiates into a replicative intracellular amastigote. Epimastigote and metacyclic trypomastigote forms have been shown in the insect vector (1). There are neither satisfactory drugs nor a vaccine against Chagas disease. New drugs should be aimed at steps in a metabolic pathway which are absent or different from analogous steps in the host. The metabolism of glucose in the Kinetoplastida, the order to which *T. cruzi* belongs, has attracted a great deal of attention in this respect, since most of the glycolytic enzymes are compartmentalized in a unique organelle termed the glycosome (2). Membrane transport processes represent potential targets, since they import vital nutrients without which the parasite would die. Glucose carriers are among the best studied transport systems, and proteins of kinetoplastids belonging to the glucose transporter superfamily are known. Both proton-motive active transporters and facilitated diffusion systems have been demonstrated in this order (reviewed in ref. 3). D-Fructose is also transported by the *T. brucei* hexose transporter (4) but not by the mammalian erythrocyte GLUT1 protein, highlighting differences between transporters in host and parasite.

We report here the cloning and characterization of a glucose transporter gene† from *T. cruzi* and the comparison of glucose transport in *T. cruzi* with the transport system in other kinetoplastids.

MATERIALS AND METHODS

Trypanosomes. *T. cruzi* strain C.L. (a gift from P. Minoprio, Pasteur Institute, Paris) trypomastigote and epimastigote forms were cultured and prepared as described (5, 6).

Genomic and cDNA Analysis. Ten thousand clones of the *T. cruzi* C.L. bacteriophage λEMBL3 genomic library (kindly provided by H. Eisen, Fred Hutchinson Cancer Research Center, Seattle) were screened at low stringency (7) with a ^{32}P-labeled cDNA (ptb1c) corresponding to the *T. brucei* THT1 glucose transporter gene (8). Sequencing was performed with a T7 sequencing kit (Pharmacia). Northern blot analysis was performed as described (8). DNA and amino acid sequences were analyzed with the DNA STRIDER and CLUSTAL programs (9), with default parameters.

cDNA was synthesized from poly(A)$^+$ RNA with a first-strand cDNA synthesis kit (Pharmacia) and then amplified directly by PCR using either oligonucleotides within the coding sequence corresponding to oligonucleotide combinations XT5′ with C4 and XT5′ with C2, or with one internal oligonucleotide C1 together with oligo(dT). Nucleotide sequences of the primers are as follows (5′ to 3′): XT5′, ATGCCATCCAAGAAGCAGAC; C4, TGCCGTTATAGGACG; C2, GCACAAAGAAGCACGGT; C1, ATGCCGTGATGAATTA. Products were separated by agarose gel electrophoresis and sequenced after cloning into plasmid pUC18.

Preparation of mRNA and Microinjection into *Xenopus* Oocytes. A PCR fragment containing the TcrHT1 coding sequence was introduced into an expression vector, BSDP1400H-A65 (kindly provided by H. B. Osborne, Centre National de la Recherche Scientifique URA256, Rennes, France), and prepared for *in vitro* transcription (10). Oocytes were prepared, mRNA was injected, and uptake of 2-deoxy-D-glucose was assessed (10). 2-Deoxy-D-glucose was used at 2 mM in uptake experiments to maintain a diffusion gradient. In order to correspond to K_i values demonstrated by Eisenthal *et al.* (11) for hexose uptake in *T. brucei*, competing sugars were used in a vast excess (250 mM). As shown in ref. 10, inhibition of transport at this concentration correlates with the K_i values determined in experiments with *T. brucei*. Relatively low concentrations must be used with trypanosomes, due to the fast rate of uptake and due to the physiological problems experienced by these organisms at high sugar concentration.

Preparation of Cells for Transport Studies. Cell cultures (*T. cruzi* epimastigote and trypomastigote forms) were centrifuged at 2000 × *g* for 10 min at 20°C. The pellet was washed twice in phosphate-buffered saline [PBS: 1.8 mM KH_2PO_4/5 mM K_2HPO_4/0.9% (wt/vol) NaCl, pH 7.4] and finally resuspended in PBS. The protein concentration of cells in the final suspension was determined by the method of Bradford (12).

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. U05588).

Transport Assay (Centrifugation Through Oil). An oil method similar to that described (13) was used to measure transport of 0.5 mM D-glucose and 2-deoxy-D-glucose at 22°C. Cell suspensions (100 μl containing 2×10^7 cells) and 100 μl of 1 mM D-glucose or 1 mM 2-deoxy-D-glucose (0.2 μCi of D-[U-^{14}C]glucose or 1 μCi of 2-deoxy-D-[1-^{3}H]glucose; 1 μCi = 37 kBq) in PBS were preincubated separately at 22°C. Cells and incubation medium were then mixed and incubated at 22°C for the required time on a cushion of 100 μl of oil [1-bromododecane, 98% (Aldrich); final density, 1.038 g/ml] and then centrifuged for 30 sec to stop the reaction. The aqueous layer was aspirated, and the surface of the oil was gently washed three times and then aspirated. The pellet was dissolved in 200 μl of 2% SDS, and the incorporated radioactivity was assayed by liquid scintillation counting.

The effect of inhibitors on D-glucose transport was studied by incubating cells with the inhibitor for 5 min prior to the commencement of the transport assay.

RESULTS

Isolation and Sequencing of a Genomic Clone from *T. cruzi*. A DNA clone containing a 15-kb insert (TcrHTg) was isolated from a genomic library by using the *T. brucei* glucose transporter gene (THT1) as a probe at low stringency. A 2.58-kb *Bam*HI fragment was subcloned into the pUC18 vector, giving plasmid pB7, which was sequenced on both strands. An open reading frame designated TcrHT1, for *T. cruzi* hexose transporter 1, was identified (Fig. 1).

Tandem Organization of Multiple Copies of TcrHT Genes and Their Expression. Partial *Eco*RV digestion of total DNA resulted in a ladder of eight fragments detected with a probe derived from the 3′ noncoding sequence downstream of TcrHT1. This suggests the presence of at least eight tandemly reiterated copies of the TcrHT gene on one of the chromosomes of *T. cruzi* C.L. (data not shown). Northern blot analysis of trypomastigote and epimastigote total RNA revealed two mRNA transcripts of equal abundance [quantification by PhosphorImager (Molecular Dynamics)] called E1 (2.6 kb) and E2 (2.1 kb) (Fig. 2*A*).

cDNAs corresponding to different transcripts were synthesized and PCR was used to amplify both the internal coding sequences (by using pairs of oligonucleotide primers within the coding sequence, indicated in *Materials and Methods*) and the 3′ noncoding regions [by using oligo(dT) and one internal primer]. All combinations of primers designed to amplify within the coding region yielded sequences identical (or with occasional single nucleotide changes) to the genomic sequence. The 3′ nontranslated regions, however, differed in two different cDNA clones (Fig. 2*B*). One clone (ETC3c) was identical to the genomic sequence in plasmid pB7 and corresponded in size to transcript E1. A second, shorter cDNA (ETC8c) presumed to correspond to transcript E2 was identical in the first 160 bp of 3′ nontranslated sequence, after which its sequence diverged. To verify that the two different 3′ sequences were derived from different intergenic regions within the TcrHT cluster, PCR amplification was performed on genomic DNA, using two gene-internal primers designed to amplify across the intergenic regions. Two products were obtained with sizes corresponding to the different 3′ nontranslated regions of ETC3c and ETC8c.

Structural Analysis of TcrHT1. Translation of the TcrHT1 sequence predicts a single long open reading frame encoding a 544-amino acid polypeptide (Fig. 1). Hydropathy profiles for the predicted primary structure of the TcrHT1 protein, calculated according to the method of Kyte and Doolittle (15), predicted 12 putative membrane-spanning domains (indicated in Fig. 1). These can be arranged with a membrane

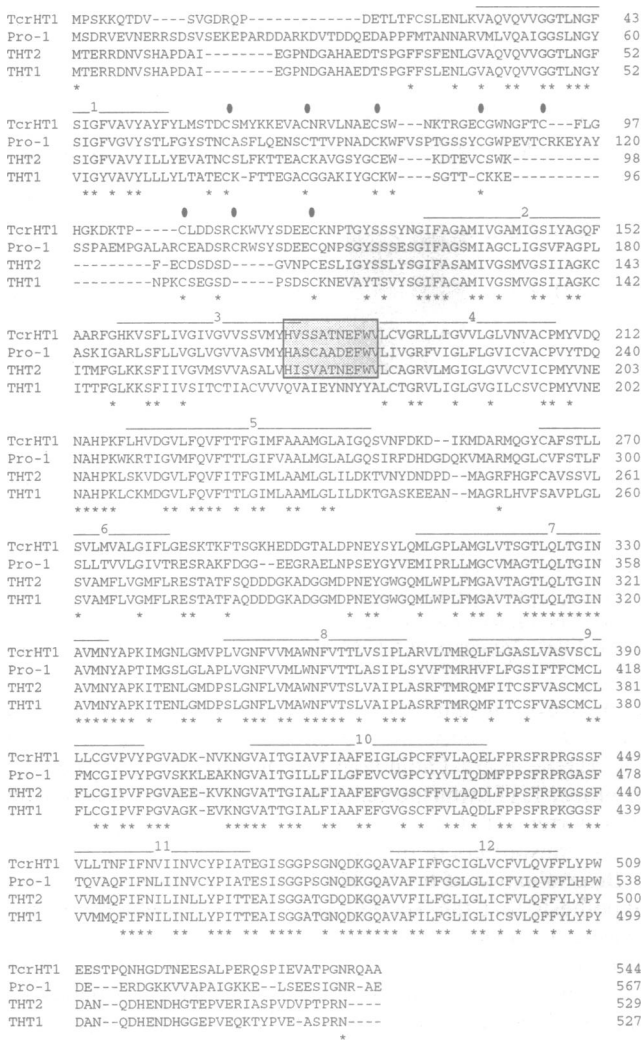
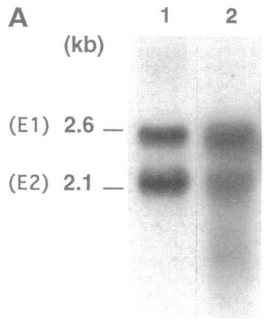


FIG. 1. Comparison of the predicted amino acid sequence of the *T. cruzi* TcrHT1 gene with the *T. brucei* THT1 and THT2 amino acid sequence (10) and the *Leishmania* Pro-1 sequence (14). Gaps were introduced to optimize the alignment; identical amino acids are indicated by stars. The 12 hydrophobic segments predicted by the method of Kyte and Doolittle (15), representing putative membrane-spanning domains, are overlined and numbered 1–12. The cysteine residues located in the first loop are indicated by filled ovals. The sequence located between the third and fourth transmembrane segments of TcrHT1 with great homology to Pro-1 and THT2 is boxed.

topology characteristic of the model for many members of the glucose transporter superfamily (16, 17). In this model, the N and C termini are located on the cytoplasmic side of the plasma membrane. An extracellular loop of 69 amino acids, rich in cysteines, is located between the first two transmembrane segments. Similar large cysteine-rich loops are found in the other kinetoplastid transporters [*T. brucei* THT1 and THT2 (10); *Leishmania* Pro-1 (14) and D2 (18)], whereas other members of the glucose transporter superfamily contain a much shorter loop. A potential N-linked glycosylation site (Asn-Lys-Thr at amino acids 81–83) in this first exofacial loop, described in several other members of the superfamily, is also present in the TcrHT1 sequence.

Optimally aligned TcrHT1 sequence shares with the *T. brucei* THT1 sequence 52% identity and 65% homology when conservative amino acid substitutions are considered. With the second *T. brucei* transporter THT2 and the *Leishmania enriettii* Pro-1 sequences, TcrHT1 shows, respectively, 55% and 54% identity with 72% and 73% homology (Fig. 1).



B

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                                                E  V  A  T  P  G  N  R  Q  A  A
ETC3c                                           GAGGTTGCCACCCCTGGCAACCGTCAAGCCGCGTGAGTGTGTCGTGTGG  13
ETC8c                                           ---------------------------------------------------  13

ETC3c  CACCGTGTGAGACGTGAGACCAATATATCTTACGGCAAAATAAAACGCAAAACGAGGGTGCAGGACTGCGTTGCTGCTGA  93
ETC8c  ---------------------------------------------------------------------------------  93

ETC3c  CGACACTGCGAAAACCGCGCTCTCCATCGCAGGTGTCGTCCGATGGGGTTGGCGCGCAGCACCCGATAGAGCCAAGCGGC 173
ETC8c  --------------------C-----C-------  ---------------------------------TAAAAATCCTT 172

ETC3c  ATCCTGCGTTTTTCTGGTTACGGGCAACTTTACTCCTTTCTGATTCTAATTGAAAACGGCTATATGTATATATATATATT 253
ETC8c  TTTTTTTTATTATTATTGTTTTCCCATTCTTTAGTAAAAATCCTTTTTTTTTTTTAATTGTTTCCCCATTCTCTTATTTA 252

ETC3c  GGGAAATTGTGAAAAGGGTGTTTGCGAGGGGGCTGGTTAACTCAATGAATATCTTGTGATAAAACAAATACATTTCTGGG 333
ETC8c  TTTTGGTTTGGTACCTCTTTTTTCTCCTTTTGTTTTTTTTTACTTTATCATTGAAAACGTTCAAAAATGTAAGAATTTTA 332

ETC3c  CGATTTTTGTTTCTTTTTCTAACTTTTGTTCTTTCGGGGGACCGCGACGGAAGGAGTCAAGGGGCCGATAGCGGTGAGAC 413
ETC8c  AATTAAAAATTTTTTGGTTGATTTTGTAGTGATTAACCCCTTGAACACCTGTTTTGTACATCTTTGAAACTTAATTGGAA 412

ETC3c  GAAATTATTTATGTATTGATTTGTTGTTTTTCCGCTCACCCCCTTTTGGTTCCTCCACTTGCGGCGGGGTCTTGTGGCTG 493
ETC8c  ATTATAA Poly A                                                                    419

ETC3c  GAGGATCCGAAAGTCTGCCACTTCTTATGTAATGAACATTTCGAATTCGAATAGAGTCCACCATTTCATGGTGGTGACTA 573

ETC3c  TCAGAATATATAGTTGGAGCAAACATCGAGGATGTGGCGAACCCACTGCCTCTCCACCATCTATTTTTTTCCCCTCCTTT 653

ETC3c  CTTGTCTGTGA Poly A                                                               664
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FIG. 2. (*A*) Northern blot of trypomastigote (lane 1) and epimastigote (lane 2) RNA, probed with the TcrHT1 gene. (*B*) Sequence alignment of the 3′ nontranslated regions of the cDNAs ETC3c and ETC8c. The stop codon of the coding sequence is underlined. Dashes represent identical nucleotides. Numbers of nucleotides after the stop codon are marked at the right side of the sequence.

Comparison of amino acid residues comprising the second exofacial loop (between transmembrane segments 3 and 4; Fig. 1) reveals great homology among TcrHT1, Pro-1, and the *T. brucei* THT2 transporter. The *T. brucei* bloodstream-form transporter THT1, however, shares little homology with the other transporters in this region.

Determination of the TcrHT Protein Function. To test the hypothesis that the TcrHT1 gene encodes a glucose transporter, we injected *in vitro* transcribed TcrHT1 RNA into *Xenopus* oocytes (19, 20) and then measured their ability to transport the nonmetabolizable glucose analogue 2-deoxy-D-glucose. Uptake was measured over 2 hr, a time during which D-glucose itself would be metabolized, hence the need for a nonmetabolizable analogue.

Oocytes injected with 10 ng of TcrHT1 RNA transport 2-deoxy-D-glucose at a markedly increased rate when compared to oocytes injected with water (data not shown). A significant increase in uptake was observed as early as 48 hr after injection, and uptake activity increased exponentially for up to 3 days, at which point uptake into oocytes expressing the TcrHT1 gene was at 210 ± 5 pmol per oocyte in 2 hr, whereas oocytes injected with water transported at only 10% of this rate. Reagents known to inhibit the transport system in *T. cruzi* were tested on the heterologously expressed TcrHT1 carrier. The uptake of 2-deoxy-D-glucose in oocytes expressing the TcrHT1 protein was inhibited 75% by a 125-fold excess of D-glucose but not by the same excess of L-glucose, suggesting that transport is stereospecific (Fig.

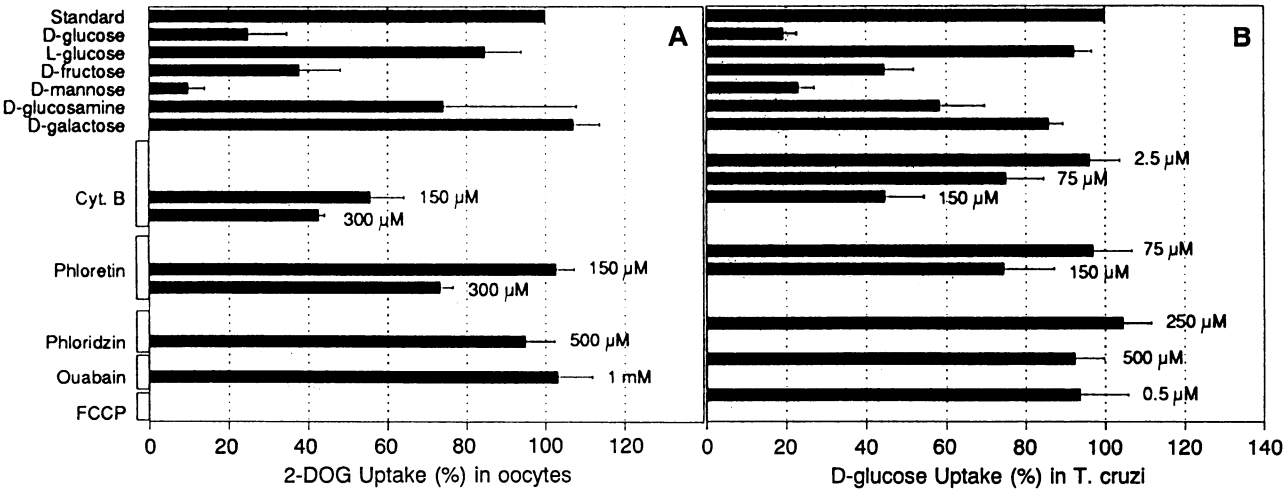

FIG. 3. Specificity of the glucose transport system and inhibition of glucose uptake. (*A*) Uptake of 2-deoxy-D-glucose (2-DOG) in *Xenopus* oocytes injected with RNA encoding TcrHT1 and incubated with competitors (sugars as indicated at 250 mM) or inhibitors (at the concentrations shown to the right of the bars). Cyt. B, cytochalasin B; FCCP, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone. Velocity of 2-DOG uptake by the oocytes was calculated and expressed as a percentage of the value for uptake without inhibitor. Each value represents the mean ± SD of three groups of 10 injected oocytes. (*B*) Effect of inhibitors on D-glucose uptake in *T. cruzi*. Potential competitors and inhibitors were preincubated as described in *Materials and Methods*. The initial rate of D-glucose transport was measured over 20 sec by using centrifugation through oil. D-Glucose transport in the absence of competitor or inhibitor was set to 100%. Values are expressed as mean ± SD (n = 4). Competing sugars were at a final concentration of 5 mM. Inhibitors were added from stock solutions in 70% (vol/vol) ethanol and cells in a parallel control experiment were incubated with the same ethanol concentration. Potential inhibitors of the three different eukaryotic glucose-transport systems were tested as in *A*.

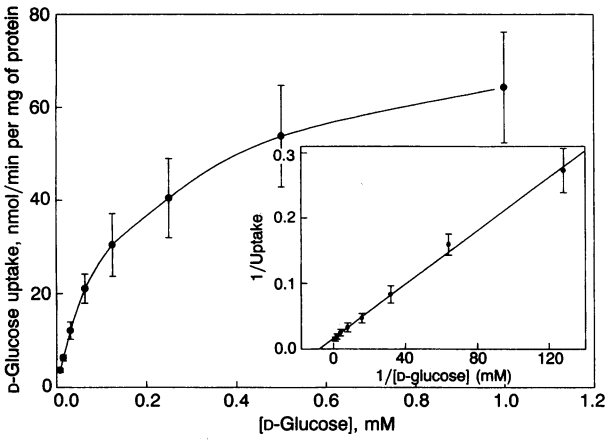

FIG. 4. Kinetics of initial D-glucose uptake. The rate of D-glucose transport was measured over the concentration range of 0.02–1 mM at 22°C. Assays were conducted by determining the initial rate of transport (fixed 10-sec time point), using centrifugation through oil. Values are expressed as mean ± SD (n = 6). (*Inset*) Double reciprocal plot.

3*A*) and that the observed inhibition does not arise from physiological phenomena related to the use of competing sugars at 250 mM. We observed significant inhibition of transport by D-fructose (62%) and D-mannose (90%), less with D-glucosamine (26%), and none with D-galactose (Fig. 3*A*). Cytochalasin B (0.3 mM) was an effective inhibitor of TcrHT1 transport (57%), as was to a lesser degree phloretin (0.3 mM) (27%). Neither phloridzin (0.5 mM) nor ouabain (1 mM) inhibited the transport (Fig. 3*A*). These results demonstrate that TcrHT1 is indeed a hexose transporter.

Kinetics of Uptake in *T. cruzi*. Significant differences were observed in the kinetics of uptake of 2-deoxy-D-glucose and D-glucose in epimastigotes (see below). The rate of transport of 2-deoxy-D-[1-^{3}H]glucose was linear for 2 min for 0.5 mM substrate (K_m = 154 ± 4 μM and V_{max} = 3.65 ± 0.33 nmol/min per mg of protein). D-[^{14}C]Glucose transport was linear for 30 sec and has a different K_m (see below). Incorporation does not reach a plateau with D-glucose because it is metabolized after uptake (data not shown). For (Zero-trans) kinetic analysis, transport of D-glucose was studied over the concentration range 0.02–1 mM at a fixed time of 10 sec, during which transport is linear. The kinetics of initial D-glucose uptake showed a saturable process typical for a carrier-mediated transport system (Fig. 4). A double-reciprocal plot of the data was well fit by a straight line (Fig. 4 *Inset*), which suggests a single transport system is responsible for D-glucose uptake within the concentration range 0.02–1 mM. The K_m for D-glucose uptake was 84.1 ± 7.9 μM and the V_{max} was 46 ± 9.4 nmol/min per mg of protein. Transport kinetics for 2-deoxy-D-glucose were also measured for bloodstream-form trypomastigotes and revealed a single transport system with a K_m of 145 ± 21 μM and V_{max} of 1.4 ± 0.1 nmol/min per mg of protein. A similar transport system therefore operates in both epimastigote and trypomastigote forms of the parasite.

Substrate Specificity of the Glucose Transport System. Fig. 3*B* shows the effect of a 10-fold excess of various sugars on the transport of 0.5 mM D-glucose. L-Glucose and D-galactose gave <15% inhibition, suggesting that these molecules do not compete for the transporter. D-Glucose, D-fructose, and D-mannose significantly inhibited (70–85%) transport, whereas D-glucosamine inhibited at 42%. Similar but not identical inhibition patterns were obtained by measuring the uptake of 2-deoxy-D-glucose in the presence of these compounds (data not shown).

Since transport kinetics of 2-deoxy-D-glucose were observed to differ significantly from those of D-glucose, we tested a number of C-2 and other glucose analogues. K_i values for D-glucose, D-mannose, 2-deoxy-D-glucose, D-glucosamine, D-fructose, and D-galactose were calculated by the method of Eisenthal *et al.* (11) at a concentration of 6 μM D-glucose from the plot of uninhibited rate divided by inhibited rate versus competitor concentration (Table 1). From these data it appears that the C-2 position is more important in the transport of hexoses by the *T. cruzi* transporter than it is for that of other kinetoplastids or the mammalian GLUT1 transporter.

Inhibitors of Glucose Uptake. The mode of action of the glucose transport system in *T. cruzi* was investigated by using a series of inhibitors specific for different glucose-uptake systems (Fig. 3*B*). D-Glucose uptake was not affected by the Na^+/K^+-ATPase inhibitor ouabain or by the Na^+–glucose symport inhibitor phloridzin, indicating Na^+-independent transport. Uptake was also unaffected by KCN (data not shown), which inhibits the respiratory chain, or by the protonophore carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone, thus eliminating the possibility of active transport or a glucose–H^+ symport. The most effective inhibitors of *T. cruzi* glucose transport were cytochalasin B (150 μM), with 55% inhibition, and phloretin (150 μM), with 26% inhibition (Fig. 3*B*). K_i values were determined for these two inhibitors (Table 1).

DISCUSSION

We report here the characterization of glucose transport in epimastigote forms of the parasitic protozoan *T. cruzi* and the cloning of a gene which, when expressed in the *Xenopus* oocyte system, encodes a protein with characteristics similar

Table 1. Inhibition constants (K_i) for D-glucose analogues and inhibitors

Compound	*T. cruzi* epimastigote K_i, mM	*T. brucei* bloodstream form K_i, mM	*T. brucei* procyclic form K_i, mM	GLUT1 K_m, mM
Analogues				
D-Glucose	0.0594 ± 0.010	0.90 ± 0.04[a]	0.045[c]	4–10[d]
D-Mannose	0.0256 ± 0.001	0.67 ± 0.10[a]	0.031[c]	7–14[d]
2-Deoxy-D-glucose	0.248 ± 0.019	0.53 ± 0.08[a]	0.038[c]*	7[e]
D-Glucosamine	1.32 ± 0.10	21.34 ± 3.68[a]	2.0[c]	—
D-Fructose	0.338 ± 0.016	2.56 ± 0.40[a]	1.5[c]	9300[d]
D-Galactose	61 ± 7.5	>250[a]	0.59[c]	12–60[d]
Inhibitors				
Cytochalasin B	0.0266 ± 0.002	0.44[b]	0.1 (41%)[c]	0.00026–0.0006[f]
Phloretin	0.0421 ± 0.010	0.064[b]	—	0.0005–0.0025[g]

The K_i values for *T. cruzi* were determined by inhibition of D-glucose uptake using 10-sec time-point assay at 22°C. Values are expressed as mean ± SD (n = 4). Other values are from refs. 11 (a), 21 (b), 22 (c), 23 (d), 24 (e), 25 (f), and 26 (g).

to those of the glucose transporter responsible for hexose uptake in *T. cruzi*.

Other members of the Kinetoplastida have been shown to possess multiple isoforms of molecules involved in glucose transport which are differentially expressed in the parasite life cycle. Two linked genes have been identified in the African trypanosome *T. brucei* (10), a situation which may correlate with separate transport systems in the bloodstream and insect forms of this parasite (11, 22). A pair of genes encoding putative hexose transport molecules have also been identified in both *Leishmania enriettii* and *Leishmania donovani* (14, 18).

Biochemical analysis of glucose transport in *T. cruzi* supports the notion that a single high-affinity plasma membrane transporter is present in the life-cycle stages studied. The intracellular replicative amastigote form of the parasite has not been studied. A second low-affinity transporter similar to that in the bloodstream form of African trypanosomes is not evident in *T. cruzi*.

The relatively high affinity for D-glucose demonstrated by the *T. cruzi* transporter is similar to that seen for other insect-form kinetoplastids (22, 27, 28), suggesting that an ancestral kinetoplastid glucose transport molecule had high affinity for glucose and that bloodstream-form trypomastigotes of *T. brucei* have evolved a second, low-affinity transport molecule as an adaptation to the glucose-rich environment of the mammalian bloodstream (29). The *T. cruzi* TcrHT1 gene sequence shares more homology with the *Leishmania* Pro-1 and *T. brucei* THT2 genes than the THT1 gene from *T. brucei*, particularly in certain regions, such as the second exofacial loop boxed in Fig. 1. The former two genes are believed to encode isoforms of the transporter expressed in insects, and the latter a bloodstream-expressed isoform.

Facilitated diffusion operates to transport glucose in bloodstream-form *T. brucei* (21) and *Crithidia* (28), whereas in *Leishmania* and procyclic-form *T. brucei*, both proton-gradient-mediated active transport (22, 27) and facilitated diffusion have been proposed in different studies (30, 31). Inhibitor analysis of the mode of action of the TcrHT protein clearly revealed a facilitated-diffusion molecule.

Significant differences in substrate binding have been noted for the *T. brucei* bloodstream-form transporter when compared to the mammalian erythrocyte transporter GLUT1 (11). Importantly the *T. brucei* transporter can recognize and transport D-fructose, unlike GLUT1 (4). The *T. cruzi* transporter also recognizes D-fructose with high affinity. The liver-type hexose transporter (GLUT2) recognizes D-fructose (32), but with a K_m of 66 mM, significantly higher than that of the kinetoplastid transporters. D-Galactose, the C-4 anomer of D-glucose, is recognized by GLUT1 and the transporter present in the procyclic form but not the bloodstream form of *T. brucei*. The carrier protein in *T. cruzi* does not recognize D-galactose.

A model for the transport of glucose in *T. brucei* (11) proposes that hydrogen bonds are not formed between the C-2 hydroxyl group of D-glucose and the transport protein. In *T. cruzi*, the available evidence suggests that hydrogen bonds are formed between the carrier protein and the C-2 hydroxyl group.

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