

Characterization of D-glucose transport in *Trypanosoma rangeli*

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SUMMARY

Like in other trypanosomatids D-glucose is a crucial source of energy to *Trypanosoma rangeli*, a non-pathogenic parasite that in Central and South America infects triatomine vectors and different mammalian species, including humans. In several trypanosome species, D-glucose transporters were already described and cloned. In this study, we characterized the D-glucose transport activity present in 2 life-stage forms of *T. rangeli* (epimastigotes and trypomastigotes) using D-[U-¹⁴C]glucose as substrate. Our results indicate that *T. rangeli* transports D-glucose with high affinity in both epimastigote (K_m 30 μ M) and trypomastigotes (K_m 80 μ M) life-forms. Both transport activities were inhibited by Cytochalasin B and Phloretin, indicating that probably D-glucose uptake in *T. rangeli* is mediated by facilitated diffusion of the sugar. Significant differences were observed between epimastigotes and trypomastigotes in relation to their affinity for D-glucose analogues, and the predicted amino acid sequence of a putative D-glucose transporter from *T. rangeli* (TrHT1) showed a larger identity with the *T. cruzi* D-glucose transporter encoded by the TrHT1 gene than with other transporters already characterized in trypanosomatids.

Key words: *Trypanosoma rangeli*, D-glucose, D-fructose, hexose transporter.

INTRODUCTION

Trypanosoma rangeli is a digenetic haemoflagellate parasite from the Herpetosoma subgenus that infects humans as well as domestic and wild animals in Central and South America. Triatomine bugs, especially those of the Rhodnius genus, are vectors of *T. rangeli* among the vertebrate hosts (D'Alessandro and Saravia, 1992). During the last few years, biological, biochemical and molecular characterization of several *T. rangeli* isolates has revealed the genomic and antigenic plasticity of this parasite, for which the life-cycle in the mammalian hosts is still unknown (Cuba, 1998; Grisard *et al.* 1999; Guhl and Vallejo, 2003).

This parasite has an overlapping distribution with *Trypanosoma cruzi*, the causative agent of Chagas disease, allowing the occurrence of single and/or mixed infections in both vertebrate and invertebrate hosts in the same geographical region. About 2600 cases of *T. rangeli* infection have been described in Latin-American countries (Vallejo *et al.* 2002).

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In Brazil, the first case was detected in 1996 in the Amazon region (Coura *et al.* 1996) and today *T. rangeli* has already been described in 7 states, showing a wide geographical distribution of the parasite (Cuba, 1998; Ramirez *et al.* 1998; Grisard *et al.* 1999). Despite its non-pathogenic characteristic in vertebrate hosts, *T. rangeli* infection presents a crossed antigenic reaction with *T. cruzi* due to homology of at least 60% of their antigens (Guhl and Vallejo, 2003). So, the differential diagnostic between the two species is very difficult and a serious problem for the diagnosis of Chagas disease, since infection with *T. rangeli* will give false-positive results.

While in the insect stage trypanosomatids can use some amino acids as a source of energy (Bursell, 1981; Ter Kuile, 1997), all parasite bloodstream forms are entirely dependent upon rapid D-glucose metabolism through glycolysis for survival. Since D-glucose transport across the plasma membrane is generally the rate-limiting step for metabolism (Ter Kuile and Opperdoes, 1991; Bakker *et al.* 1999), the study of D-glucose transport in trypanosomatids has received considerable attention as the plasma membrane D-glucose transporters can be potential drug targets for chemotherapy (Barrett *et al.* 1998; Tetaud *et al.* 1997; Szablewski, 2000; Bayele, 2001; Azema *et al.* 2004).

In several trypanosome species (*T. brucei*, *T. congolense*, *T. cruzi*, *T. equiperdum*, *T. rhodesiense*; *T. b. gambiense* and *T. vivax*) D-glucose transporters have been already cloned and described (Bringaud and Baltz, 1994; Barrett *et al.* 1998; Tetaud *et al.* 1997; Szablewski, 2000), and they all belong to the Major Facilitator Superfamily of transporters with high amino acid sequence homology among them (52–80% identity). While in some species 2 isoforms with different affinities can be found, in other parasites only 1 form has been described, which may reflect an adaptation to the different environments to which these parasites are exposed. For example, *T. brucei* and *T. congolense* have 2 different glucose transporter isoforms (Gruenberg *et al.* 1978; Munoz-Antonia *et al.* 1991; Vendrenne *et al.* 2000) that allow these parasites to develop in both the mammalian bloodstream (with D-glucose concentrations in the 5 mM range) and also the insect vector (with a far lower abundance of D-glucose). A low affinity transporter (K_m 10^{-3} – 10^{-4} M), denominated THT1 (or TcoHT1 for *T. congolense*), is highly expressed in bloodstream forms, while a high affinity isoform (K_m 10^{-5} – 10^{-6} M), denominated THT2, is predominantly expressed in *T. brucei* procyclic forms. The high affinity TcoHT2 transporter is expressed in all the developmental stages of the parasite (Bringaud and Baltz, 1993, 1994; Barrett *et al.* 1998; Vendrenne *et al.* 2000). The *T. brucei* THT transporters are 80% identical, with the genes arranged in tandem repeats on 2 homologue chromosomes with 6 copies of THT1 and 5 copies of THT2. Both *T. congolense* TcoHT isoforms are 92.4% identical, and encoded by a single cluster of genes containing 2 copies of TcoHT1 and 3 copies of TcoHT2 arranged alternately.

A different situation is found in *T. cruzi* where a single D-glucose transporter isoform of moderated affinity (K_m 10^{-4} – 10^{-5} M) is expressed at similar levels in epimastigote and trypomastigote forms, indicating that provably the molecular properties of this transporter are sufficient to allow the survival of the parasite in D-glucose-poor environments (insect vector and intracellularly in the mammalian host). This parasite has 8 tandem copies of the TcrTH1 gene in 1 chromosome, but Northern blot analysis revealed 2 transcripts that differ in size, a finding with still unknown function (Tetaud *et al.* 1994). *T. vivax*, which only transiently stays in the insect vector, has only a single low affinity isoform and the gene (TvHT1) occurs as a cluster of at least 6 tandemly repeated identical copies (Waitumbi *et al.* 1996).

Since little is known concerning the basic biochemistry of carbohydrate transport and metabolism by *T. rangeli*, in the present study we describe the characterization of D-glucose transport in different life-stage forms of the parasite, and isolate a partial DNA sequence of a putative D-glucose transporter gene from *T. rangeli*.

MATERIALS AND METHODS

Parasites

The *T. rangeli* Choachi strain, isolated from naturally infected *Rhodnius prolixus* in Colombia (Schottelius, 1987), was used in the present study. The epimastigote forms were cultivated at 27 °C in liver-infusion tryptose (LIT) medium supplemented with 10% of fetal bovine serum, 100 µg/ml of streptomycin and 100 units/ml of penicillin. The trypomastigotes forms were obtained by cultivating epimastigotes in DMEM medium, pH 8.0 at 28 °C, according to Koerich *et al.* (2002).

Transport assays

The transport assay was adapted from a method used to characterize L-proline transport in *T. cruzi* (Silber *et al.* 2002). *T. rangeli* epimastigote and trypomastigote forms were centrifuged at 2000 g for 10 min at 25 °C. The pellet cells were washed 3 times in phosphate-buffered saline (PBS) pH 7.4, re-suspended in the same buffer, and conserved in an ice bath until use. Approximately 50 µl of epimastigotes (5×10^6 cells) or trypomastigotes (1×10^7 cells) were placed in a microcentrifuge tube, incubated for 5 min at room temperature, and the reaction started by addition of 10 µl of different glucose concentrations in the presence of 0.2–0.5 µCi of D-[U-¹⁴C]glucose (Amersham Bioscience Corp.). After 5 sec at room temperature, the uptake of the sugar was interrupted by the addition of 1 ml of cold 100 mM D-glucose in PBS containing 100 µM HgCl₂, followed by a short (>1 min) centrifugation and 1 additional wash in the same buffer. The radioactivity incorporated into the cells was determined by liquid scintillation, and negative controls with previously boiled parasites were used.

Competition assays were performed with 0.3 mM D-glucose containing 1 µCi of D-[U-¹⁴C]glucose in the presence of the several analogues (D-mannose, 2-deoxy-D-glucose, D-glucosamine, D-galactose, D-fructose) at a final concentration of 3 mM. Inhibition assays were performed by pre-incubating the parasites for 5 min at 25 °C with 150 µM cytochalasin B or phloretin, or with 5 mM phloridzin. After pre-incubation, transport assays were performed using 0.3 mM D-glucose containing 1 µCi of D-[U-¹⁴C]glucose. For the calculation of K_i a concentration of 0.01 mM D-glucose containing 1 µCi of D-[U-¹⁴C]glucose was used with sugar analogues at a concentration of 0.05, 0.1, and 0.5 mM, and transport inhibitors at a concentration of 75, 150, and 200 µM for cytochalasin B and phloretin, or 1, 5, and 10 mM of phloridzin. Apparent K_i values were determined using the equation $v_0/v = 1 + [I]/K_i$, where v_0 and v are uninhibited and inhibited transport rates, respectively, and [I] is the inhibitor concentration (Eisenthal *et al.* 1989).

Statistical analysis

All experiments were performed in triplicate. The mean and standard error of at least 3 distinct experiments were determined.

Isolation of a *T. rangeli* partial DNA sequence encoding a D-glucose transporter

In order to clone a *T. rangeli* D-glucose transporter gene, we used degenerate oligonucleotides corresponding to conserved amino acid sequences found in other trypanosomatid hexose transporters (Barrett *et al.* 1998). Initially, a 1.1 kb DNA fragment was obtained with primer THXT1-F (5' ATGATYG-TTGGYTCRATGATTGCSTCG 3'), corresponding to amino acids MIVGS/AMV/IGSI present in the middle of the predicted second transmembrane (TM-2) helix, and primer THXT2-R (5' CTG-CAGMACGAAGCARAYMARRCC 3') that corresponds to amino acids GL/IV/ICFVLQ present in the middle of the last transmembrane helix (TM-12). The PCR reaction was performed in a 25 μ l volume containing 0.5 μ l dNTP 10 mM, 1 μ l of 0.5 mM of each primer, 40 ng of phenol-buffer purified *T. rangeli* epimastigote total genomic DNA, and 1 unit of *Taq* DNA polymerase (Invitrogen-USA). The reaction tube was subjected to pre-denaturation for 2 min at 95 °C and for 25 cycles: denaturation for 1 min at 95 °C; annealing for 1 min and 10 sec at 45 °C, and elongation for 2 min at 72 °C. A final 10 min extension at 72 °C was included. The 1.1 kb fragment was purified following agarose gel electrophoresis with a Concert[®] Rapid gel extraction kit (Invitrogen-USA), cloned into pGEM-T easy[®] vector (Promega-USA), and sequenced by DYEnamic[™] ET Dye Terminator (Amersham Bioscience Corp) using T7 sequencing primers and THXT3-F (5' ACGTTTGGCAT-TATGTTTGGCGGCGGCG 3') and THXT4-R (5' CTGCTGGCTCAGCACAAAAAGCACGG 3'). Nucleotide sequence obtained from 5 independent clones were identical, and the high homology to TcrTH1 (see Results section above) allowed the extension of the sequence into TM-1 by further cloning a 315 bp DNA fragment using primers THXT5-R (5' CACCAGAAAGCTCACCTTGTGGCCAAA 3') and THXT6-F (5' TTTTGCAGCCTGCAGAA-CCTGAAGGTG 3'), corresponding to amino acids 22 to 30 of the TcrTH1 permease).

RESULTS

In order to characterize the D-glucose uptake systems present in *T. rangeli*, D-glucose transport was analysed in epimastigote and trypomastigote forms of the Choachi strain using zero-trans kinetic assays. For kinetic analysis, D-glucose transport assays were performed at a fixed incubation time of 5 sec, during which the rate of uptake was linear over a

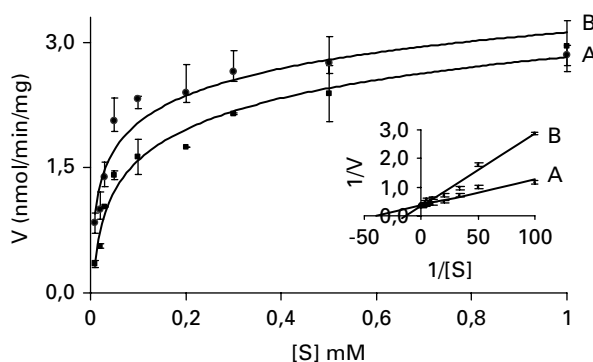


Fig. 1. Kinetics of initial D-glucose uptake by *Trypanosoma rangeli* epimastigote (A) and trypomastigote (B) forms. In both forms the rate of D-glucose uptake was measured over a concentration range of 0.01–1 mM at 25 °C. Assays were conducted by determining the initial rate of transport (fixed 5-sec time-point) using zero-trans uptake. Values are expressed as mean \pm s.d. ($n=3$). Insert, Lineweaver-Burk plot.

concentration range of 0.01–1 mM. As shown in Fig. 1 the two parasite forms exhibit a saturable process typical for a carrier-mediated transport system. A Lineweaver-Burk plot of the data suggests a single transport system with similar V_{max} (~ 2.6 nmol/min/mg) and a high affinity for D-glucose with K_m values of 30 μ M for epimastigotes and of 80 μ M for trypomastigotes, respectively.

Several D-glucose analogues were used in competition experiments in order to study the substrate specificity of the transporter present in both forms of *T. rangeli*. Fig. 2 shows the effects of a 10-fold excess of some monosaccharides on the transport of 0.3 mM D-glucose, and Table 1 gives a summary of the K_i values for each sugar. No significant inhibition by D-galactose was observed in both parasite forms, indicating that modification in C-4 allows D-glucose binding to the transporter. The position of less importance seems to be C-2, because D-glucosamine and 2-deoxy-D-glucose inhibited the transport with values close to D-glucose. In the case of D-fructose and D-mannose significant differences were observed between epimastigotes and trypomastigotes, and while these two sugars did not inhibit D-glucose uptake by epimastigotes, in trypomastigotes both sugars inhibited significantly the transporter present in this parasite stage. Significant inhibition of D-glucose uptake in *T. rangeli* forms was also observed with cytochalasin B, phloretin, and to a lower extent, also with phloridzin (Fig. 2 and Table 1).

Using PCR and degenerated oligonucleotides corresponding to conserved sequences present in other trypanosomatids high-affinity D-glucose transporters (see Fig. 3 and Materials and Methods section), we next isolated a 1.1 kb DNA fragment that encoded a protein with significant similarity to these transporters, especially the *T. cruzi* TcrHT1 high-affinity permease. Indeed, by use of a sequence

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

(The values of K_i for *T. rangeli* forms were determined by the inhibition of D-glucose incorporation as described in the Materials and Methods section. Other values are from references: (a) (Tetaud *et al.* 1994); (b) (Tetaud *et al.* 1997) and (c) (Vendrene *et al.* 2000).)

Compound	<i>T. rangeli</i> epimastigote K_i , mM	<i>T. rangeli</i> trypomastigote K_i , mM	<i>T. cruzi</i> epimastigote ^a K_i , mM	<i>T. brucei</i> procyclic ^b K_i , mM	<i>T. congolense</i> procyclic ^c K_i , mM
Analogue					
2-deoxy-D-glucose	0.77	0.24	0.248	0.038	0.34
D-glucose	0.47	0.29	0.0594	0.045	0.80
D-mannose	0.72	0.3	0.0256	0.031	0.86
D-glucosamine	1.07	1.38	1.32	2.0	4.51
D-galactose	4.8	1.72	61	0.59	33.7
D-fructose	5.6	0.39	0.338	1.5	5.70
Inhibitor					
Cytochalasin B	0.302	0.118	0.0266	N.D.	N.D.
Phloretin	0.260	0.320	0.0421	N.D.	N.D.
Phloridzin	3.4	7.3	N.D.	N.D.	N.D.

N.D., Not determined.

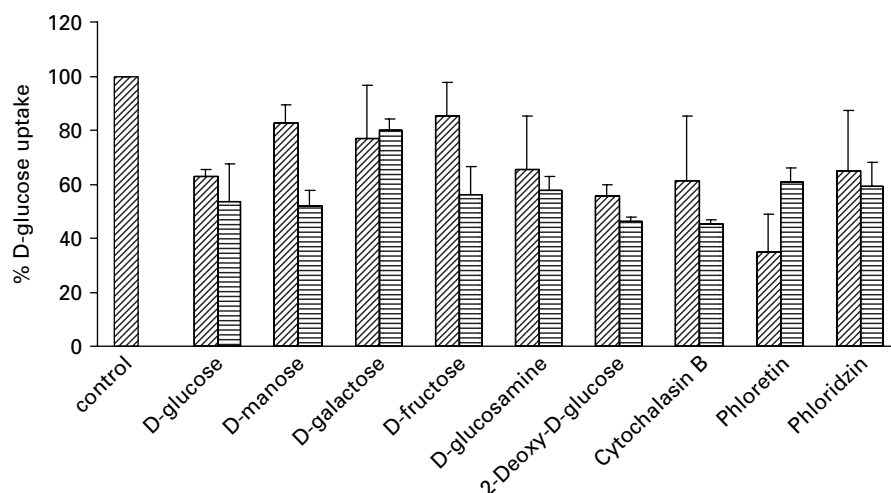


Fig. 2. Specificity and inhibition of D-glucose uptake by *Trypanosoma rangeli* epimastigotes (▨) and trypomastigotes (■) forms. The inhibition of 0.3 mM D-[U-¹⁴C]glucose uptake by unlabelled analogues (3 mM), or by the inhibitors of eukaryotic D-glucose-transport systems cytochalasin B and phloretin (both at 150 μ M) or phloridzin (5 mM), was determined with the final concentrations indicated for each compound as described in the Materials and Methods section. D-Glucose transport in the absence of analogues and inhibitors was set to 100%. Values are expressed as mean \pm s.d. ($n=3$).

corresponding to amino acids close to the first TM from the TcrHT1 gene we could extend the cloned sequence further, totaling 457 amino acids (approximately 82–85% of a full-length permease). While the DNA sequence showed a high homology with *T. vivax* (87%) and *T. cruzi* (83%) high-affinity hexose transporter genes (and \sim 35% homology with the other THT2 transporters), the amino acid identity was higher (64%) with *T. cruzi* TcrHT1, while with the permeases of other trypanosomatids values of 37–43% identity were obtained (Fig. 4). We have also used the cloned sequence to search the ongoing EST's database from *T. rangeli* (Snoeijer

et al. 2004), but unfortunately no sequence corresponding to the cloned DNA was found.

DISCUSSION

Kinetic analysis of D-glucose transport by *T. rangeli* epimastigotes and trypomastigotes indicates that a high-affinity transporter is present in both life-cycle stages studied, a situation similar to that found in other insect-form kinetoplastids including *Crithidia luciliae* and some *Leishmania* species (Knodler *et al.* 1992; Langford *et al.* 1994; Burchmore and Hart, 1995; Tetaud *et al.* 1997), and with no evidence of a

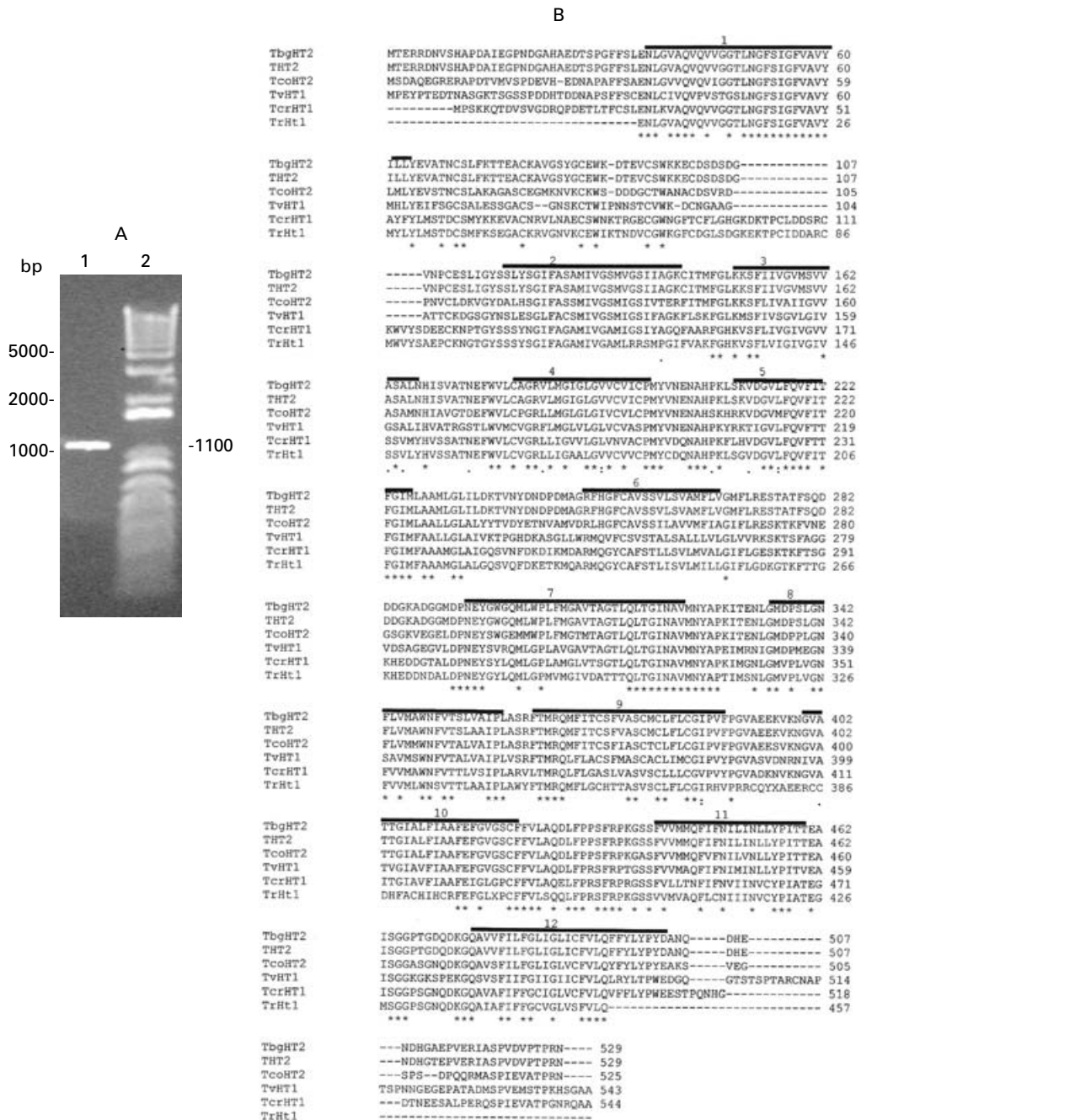


Fig. 3. (A) PCR amplification of a TrHT1 fragment. Lane 1, 1 kb Plus DNA Ladder (Invitrogen). Lane 2, PCR product. The gene was amplified from *Trypanosoma rangeli* epimastigote genomic DNA using the primers described in the Materials and Methods section. (B) Amino acid sequence alignment of the trypanosomatid D-glucose transporters. Identical amino acids are underscored with asterisk, and the 12 putative transmembrane segments are indicated with their corresponding number above.

Phylogram

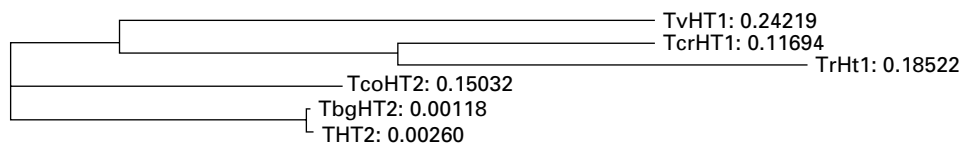


Fig. 4. Relationship between TrHT1 and other trypanosomatid hexose transporters. Sequences were retrieved from GenBank, and the phylogram was obtained using the PHYLIP package. TbgHT2 – *T. brucei gambiense* hexose transporter.

low-affinity transporter similar to that found in bloodstream forms of other trypanosomes (Tetaud *et al.* 1997; Barrett *et al.* 1998; Szablewski, 2000). Inhibitor analysis of the mode of D-glucose transport indicates that facilitated diffusion is probably the mechanism responsible for sugar uptake. Although inhibition by phloridzin could indicate an active transport system (e.g. a Na⁺/glucose symporter), this inhibitor has also been shown to interfere with the facilitated diffusion of sugars (Tetaud *et al.* 1997), and thus the use of other inhibitors (e.g. ionophores, uncoupling agents and/or H⁺-ATPase inhibitors) may be more informative on the exact mechanism of D-glucose uptake by *T. rangeli*. As is the case for other trypanosomatid transporters, the *T. rangeli* permease seems not to recognize D-galactose, and probably transports D-fructose and other sugars modified at C-2, a characteristic shared with TcrHT1 and clearly different from other mammalian (e.g. GLUT1) permeases (Barrett *et al.* 1998). Indeed, the partial DNA sequence that we have obtained from *T. rangeli* shows significant identity with the moderated-affinity D-glucose transporter TcrHT1 from *T. cruzi*. Although the results are preliminary and await further functional characterization that this gene is indeed a D-glucose transporter, and that more than one permease isoform is present in *T. rangeli* (as suggested by the differential inhibition with D-fructose and D-mannose observed in epimastigote and trypomastigote forms), the results presented here may explain some important aspects of the biology of this parasite.

For example, the lack of evidence for a low-affinity (and high capacity) D-glucose transporter in *T. rangeli*, like those found in bloodstream forms of other trypanosomes, indicates that this parasite is probably not adapted to this D-glucose rich environment, justifying its non-pathogenic status in the mammalian hosts. Another possibility is that the parasite may stay just transiently in the blood before invading some cell, as already described by some authors that found amastigote forms in cell tissues and in fibroblast cultures (Urdaneta-Morales and Tejero, 1986; Osorio *et al.* 1995), although this finding is still controversial.

In conclusion, our results on D-glucose transport by *T. rangeli* cells describe a new pattern of sugar uptake in trypanosomatids: a parasite transmitted by bite that lacks 2 transporter isoforms as observed for a typical salivarian species, but presenting just a high-affinity transporter typical of the stercoraria group. Other biochemical characterizations have produced further conflicting information on the *T. rangeli* phylogeny. In a study of β -tubulin gene sequences on a limited number of isolates, it was suggested that *T. rangeli* was more closely related to *T. brucei* than to *T. cruzi* (Amorim *et al.* 1993). However, a recent study (de Santa Izabel *et al.* 2004) detected in *T. rangeli* proteolytic enzymes from the

cysteine-proteinase group only, enzymes that have close homology to the major cysteine-proteinase of *T. cruzi*, cruzipain. Thus, despite intensive discussions on taxonomic position and evolutionary relationships, *T. rangeli* is probably a phylogenetic link between the Stercoraria and the Salivaria trypanosomatids (Grisard, 2002).

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REFERENCES

- Amorim, M. I., Momen, H. and Traub-Cseko, Y. M.** (1993). *Trypanosoma rangeli*: sequence analysis of beta-tubulin gene suggests closer relationship to *Trypanosoma brucei* than to *Trypanosoma cruzi*. *Acta Tropica* **53**, 99–105.
- Azema, L., Claustre, S., Alric, I., Blonski, C., Willson, M., Perie, J., Baltz, T., Tetaud, E., Bringaud, F., Cottlem, D., Opperdoes, F. R. and Barrett, M. P.** (2004). Interaction of substituted hexose analogues with the *Trypanosoma brucei* hexose transporter. *Biochemical Pharmacology* **67**, 459–467.
- Bakker, B. M., Walsh, M. C., Ter Kuile, B. H., Mensonides, F. I., Michels, P. A., Opperdoes, F. R. and Westerhoff, H. V.** (1999). Contribution of glucose transport to the control of the glycolytic flux in *Trypanosoma brucei*. *Proceedings of National Academy of Sciences, USA* **96**, 10098–10103.
- Barrett, M. P., Tetaud, E., Seyfang, A., Bringaud, F. and Baltz, T.** (1995). Functional expression and characterization of the *Trypanosoma brucei* procyclic glucose transporter, THT2. *The Biochemical Journal* **312**, 687–691.
- Barrett, M. P., Tetaud, E., Seyfang, A., Bringaud, F. and Baltz, T.** (1998). Trypanosome glucose transport. *Molecular and Biochemical Parasitology* **91**, 195–205.
- Bayele, H. K.** (2001). Triazinyl derivatives that are potent inhibitors of glucose transport in *Trypanosoma brucei*. *Parasitology Research* **87**, 911–914.
- Bringaud, F. and Baltz, T.** (1993). Differential regulation of two distinct families of glucose transporter genes in *Trypanosoma brucei*. *Molecular and Cellular Biology* **13**, 1146–1154.
- Bringaud, F. and Baltz, T.** (1994). African trypanosome glucose transporter genes: Organization and evolution of a multigene family. *Molecular Biology and Evolution* **11**, 220–230.
- Burchmore, R. J. S. and Hart, D. T.** (1995). Glucose transport in amastigotes and promastigotes of *Leishmania mexicana mexicana*. *Molecular and Biochemical Parasitology* **74**, 77–86.
- Bursell, E.** (1981). The role of proline in energy metabolism. In *Energy Metabolism in Insects* (ed. Downer, R. G. H.), pp. 135–154. Plenum Press, New York.
- Coura, J. R., Fernandes, O., Arboleda, M., Barrett, T. V., Carrara, N., Degraeve, W. and Campbell, D. A.** (1996). Human infection by *Trypanosoma rangeli* in Brazilian Amazon. *Transactions of Royal Society of Tropical Medicine and Hygiene* **90**, 278–279.

- Cuba, C. A. C.** (1998). Revisión de los aspectos biológicos y diagnósticos del *Trypanosoma (Herpetosoma) rangeli*. *Revista da Sociedade Brasileira de Medicina Tropical* **31**, 207–220.
- D'Alessandro, A. and Saravia, N. G.** (1992). *Trypanosoma rangeli*. In *Parasitic Protozoa* (ed. Kreier, J. P. and Baker, J.), pp. 1–54. Academic Press, Oxford.
- De Santa Izabel, A., Vermelho, A. B. and Branquinha, M. H.** (2004). Proteolytic activities in *Trypanosoma rangeli* and stercorarian trypanosomes: taxonomic implications. *Parasitology Research* **94**, 342–348.
- Eisenthal, R., Game, S. and Holman, G. D.** (1989). Specificity and kinetics of hexose transport in *Trypanosoma brucei*. *Biochimica et Biophysica Acta* **985**, 81–89.
- Grisard, E. C., Steindel, M., Guarneri, A. A., Eger-Mangrich, I., Campbell, D. A. and Romanha, A. J.** (1999). Characterization of *Trypanosoma rangeli* strains isolated in Central and South America: An overview. *Memórias do Instituto Oswaldo Cruz* **94**, 203–209.
- Grisard, E. C.** (2002). Salivaria or Stercoraria? The *Trypanosoma rangeli* dilemma. *Kinetoplastid Biology and Disease* **1**, 5.
- Gruenberg, J., Sharma, P. R. and Deshusses, J.** (1978). D-Glucose transport in *Trypanosoma brucei*. *European Journal of Biochemistry* **89**, 461–469.
- Guhl, F. and Vallejo, G. A.** (2003). *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920 – An update review. *Memórias do Instituto Oswaldo Cruz* **98**, 435–442.
- Knodler, L. A., Schofield, P. J. and Edwards, M. R.** (1992). Glucose transport in *Crithidia luciliae*. *Molecular and Biochemical Parasitology* **52**, 1–14.
- Koerich, L. B., Emanuelle-Neto, P., Santos, K., Grisard, E. C. and Steindel, M.** (2002). Differentiation of *Trypanosoma rangeli*: high production of infective trypomastigotes forms in vitro. *Parasitology Research* **88**, 21–25.
- Langford, C. K., Burchmore, R. J. S., Hart, D. T., Wagner, W. and Landfear, S. M.** (1994). Biochemistry and molecular genetics of *Leishmania* glucose transporters. *Parasitology* **108** (Suppl.), S73–S83.
- Munoz-Antonia, T., Richards, F. F. and Ullu, E.** (1991). Differences in glucose transport between bloodstream and procyclic forms of *Trypanosoma brucei rhodesiense*. *Molecular and Biochemical Parasitology* **47**, 73–82.
- Osorio, Y., Travi, B., Palma, G. I. and Saravia, N. G.** (1995). Infectivity of *Trypanosoma rangeli* in a promonocytic mammalian cell line. *Journal of Parasitology* **81**, 687–693.
- Ramirez, L. E., Machado, M. I., Maywald, P. G., Matos, A., Chiari, E. and Silva, E. L.** (1998). First evidence of *Trypanosoma rangeli* in the southeast of Brazil, an endemic region for Chagas' disease. *Revista da Sociedade Brasileira de Medicina Tropical* **31**, 99–102.
- Silber, A. M., Tonelli, R. R., Martinelli, M., Colli, W. and Alves, M. J. M.** (2002). Active transport of L-proline in *Trypanosoma cruzi*. *Journal of Eukaryotic Microbiology* **49**, 441–446.
- Snoeijer, C. Q., Picchi, G. F., Dambros, B. P., Steindel, M., Goldenberg, S., Fragoso, S. P., Lorenzini, D. M. and Grisard, E. C.** (2004). *Trypanosoma rangeli* Transcriptome Project: Generation and analysis of expressed sequence tags. *Kinetoplastid Biology and Disease* **3**, 1.
- Szablewski, L.** (2000). Facilitated hexose diffusion in kinetoplastida. *Acta Protozoologica* **39**, 183–189.
- Ter Kuile, B. H.** (1997). Adaptation of metabolic enzyme activities of *Trypanosoma brucei* promastigotes to growth rate and carbon regimen. *Journal of Bacteriology* **179**, 4699–4705.
- Ter Kuile, B. H. and Opperdoes, F. R.** (1991). Glucose uptake by *Trypanosoma brucei*. Rate-limiting steps in glycolysis and regulation of the glycolytic flux. *Journal of Biological Chemistry* **266**, 857–862.
- Tetaud, E., Bringaud, F., Chabas, S., Barrett, M. P. and Baltz, T.** (1994). Characterization of glucose transport and cloning of a hexose transport gene in *Trypanosoma cruzi*. *Proceedings of the National Academy Sciences, USA* **91**, 8278–8282.
- Tetaud, E., Barrett, M. P., Bringaud, F. and Baltz, T.** (1997). Kinetoplastid glucose transporters. *The Biochemical Journal* **325**, 569–580.
- Urdaneta-Morales, S. and Tejero, F.** (1986). *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920. Intracellular amastigote stages of reproduction in white mice. *Revista do Instituto de Medicina Tropical de São Paulo* **28**, 166–169.
- Vallejo, G. A., Guhl, F., Carranza, J. C., Lozano, L. E., Sánchez, J. L., Jaramilo, J. C., Galtero, D., Castaneda, N., Silva, J. C. and Steindel, M.** (2002). kDNA markers define two major *Trypanosoma rangeli* lineages in Latin-America. *Acta Tropica* **81**, 77–82.
- Vedrenne, C., Bringaud, F., Barret, M. P., Tetaud, E. and Baltz, T.** (2000). The structure-function relationship of functionally distinct but structurally similar hexose transporters from *Trypanosoma congolense*. *European Journal of Biochemistry* **267**, 4850–4860.
- Waitumbi, J. N., Tetaud, E. and Baltz, T.** (1996). Glucose uptake in *Trypanosoma vivax* and molecular characterization of its transporter gene. *European Journal of Biochemistry* **237**, 234–239.