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Functional Expression of Two Glucose Transporter Isoforms from the Parasitic Protozoan *Leishmania enriettii**

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The parasitic protozoan *Leishmania enriettii* contains a family of tandemly repeated genes, designated Pro-1, that encode proteins with significant sequence similarity to mammalian facilitative glucose transporters. Pro-1 mRNAs are expressed almost exclusively in the promastigote or insect stage of the parasite life cycle. The Pro-1 tandem repeat encodes two isoforms of the putative transporter, iso-1 and iso-2, which have identical predicted amino acid sequences except for their NH_2-terminal hydrophilic domains. We have now expressed both iso-1 and iso-2 by microinjecting their RNAs into *Xenopus* oocytes and assaying these oocytes for transport of various radiolabeled ligands. Both iso-1 and iso-2 transport [^{3}H]2-deoxy-D-glucose, confirming that each protein is a bona fide glucose transporter. Each isoform also transports fructose and, to a much lesser degree, mannose. Compounds which inhibit 2-deoxy-D-glucose transport in *L. enriettii* promastigotes also inhibit transport in the microinjected oocytes expressing each isoform, indicating that the substrate specificities and pharmacological properties of each isoform are similar to those measured for 2-deoxy-D-glucose transport in intact parasites. The K_m for transport of 2-deoxyglucose in oocytes expressing iso-1 is similar to that for oocytes expressing iso-2. These results reveal that both transporter isoforms have closely related functional properties and that the difference in their structures may serve some other purpose such as differential subcellular localization.

Leishmania are parasitic protozoa of the order Kinetoplastida that have two major developmental stages in their life cycle (1). Promastigotes are flagellated extracellular organisms which live in the midgut of the sandfly vector, and amastigotes are non-flagellated organisms that are adapted for survival within the phagolysosomes of the vertebrate host macrophages. Glucose is a major source of metabolic energy in the promastigotes (2), and this sugar is also metabolized, albeit at a lower level, in the amastigotes (3–5). The kinetic and biochemical properties of glucose transport by promastigotes of *Leishmania donovani* has been investigated previously (6). Transport of 2-deoxy-D-glucose (2-DOG)[1] occurs by a carrier-mediated system with high affinity for the substrate. Other hexoses such as fructose, mannose, glucosamine, *N*-acetylglucosamine, and galactose can inhibit transport of 2-DOG with various affinities (7), and some or all of these compounds may also be substrates for promastigote glucose transporters. A body of experimental evidence suggests that promastigotes may accumulate glucose via an active proton symporter that utilizes an electrochemical gradient to concentrate the sugar within the parasite (8). However, this conclusion has recently been challenged on the basis of different experiments involving growth of promastigotes at defined glucose concentrations within a chemostat (9, 10). The latter authors have contended that glucose transport probably occurs via a facilitated transport mechanism.

In a previous report (11) we have cloned a gene from *Leishmania enriettii* which is related in sequence and putative secondary structure to mammalian glucose transporters. This gene is expressed almost exclusively in the promastigote stage of the parasite life cycle and has been designated the Pro-1 gene. The Pro-1 sequences are arranged as a family of ~7–8 tandemly repeating units within the parasite genome (12). Sequencing of the initial unit and of an internal unit of this repeat revealed that the first copy in the repeat encodes a unique isoform (13) of the Pro-1 protein (iso-1). This unique isoform contains a distinct NH_2-terminal domain compared to the other members of the repeat (iso-2). Hence, iso-1 and iso-2 form modular structures containing unrelated amino-terminal sequences attached to the remainder of the protein which is apparently identical in sequence. Furthermore, these distinct NH_2-terminal segments appear to constitute separate structural domains, corresponding exactly to the hydrophilic tail that is located on the cytoplasmic surface of the plasma membrane in mammalian facilitated glucose transporters (14, 15) (Fig. 1).

A question of great importance regarding the Pro-1 proteins concerns their biochemical function. Although these proteins are related in sequence to other known transporters, they have not previously been functionally expressed to demonstrate conclusively that they are transporters nor has their substrate specificity been determined. Since various members of the glucose transporter superfamily (16) transport different substrates, membership in this family based upon sequence homology alone cannot predict the substrate(s) recognized by the protein. We have now expressed the genes encoding both iso-1 and iso-2 of Pro-1 in *Xenopus* oocytes and demonstrated that they are genuine glucose transporters. Functional characterization of their transport activities indicates that their biochemical and kinetic properties are very similar to each other and that any distinctions in their biological roles are unlikely to be related to specific transport characteristics.

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[1] The abbreviations used are: 2-DOG, 2-deoxy-D-glucose; DCCD, *N'*,*N'*-dicyclohexylcarbodiimide, FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

EXPERIMENTAL PROCEDURES

Materials—Cytochalasin B, *N'*,*N'*-dicyclohexylcarbodiimide (DCCD), carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), phloretin, phloridzin, and all sugars used as substrates or inhibitors were from Sigma. Radiolabeled sugars were purchased from DuPont-NEN. DNA polymerase from *Thermus aquaticus* (Taq polymerase) was obtained from Promega.

Parasite Culture and Transport Assays—Promastigotes of *L. enriettii* were cultured at 27 °C in Dulbecco's modified Eagle's-L medium (17) containing 10% bovine embryonic fluid (Sigma). For transport assays, parasites were pelleted, washed twice in phosphate-buffered saline, pH 7.4, and resuspended in phosphate-buffered saline at a concentration of 5×10^8 cells ml^{-1}. Cells (100 μl) were then layered over 100 μl of 96% silicone oil, 4% paraffin oil (Sigma) in a microcentrifuge tube. Transport assays were initiated by adding to the parasites radiolabeled substrate (usually [^{3}H]2-deoxy-D-glucose at 3×10^4 μCi $mmol^{-1}$) at the appropriate concentration. The suspension was then incubated for 60 s at ambient temperature, and the cells were pelleted by centrifugation at 16,000 × *g* for 30 s. The aqueous phase was aspirated, the interface was washed twice with 500 μl of water, the oil was aspirated, and the cell pellet was resuspended in 100 μl of 0.1% Triton X-100. Each sample was mixed with 2 ml of Bio-Safe II fluor (Research Products Int.) and counted on a liquid scintillation counter.

Expression of iso-1 and iso-2 in Xenopus Oocytes—For expression in *Xenopus* oocytes, the protein coding regions of iso-1 and iso-2 were subcloned into the *Eco*RI site of the oocyte expression vector pL2–5 (18), which contains *Xenopus* β-globin 5'- and 3'-untranslated regions to optimize expression levels in oocytes. Synthetic RNA was transcribed *in vitro* in a 50-μl reaction containing 40 mM Tris, pH 7.5, 10 mM NaCl, 6 mM $MgCl_2$, 2 mM spermidine, 500 μM rNTPs, 2.5 mM cap analogue $m^7G(5')ppp(5')G$ (Pharmacia Biotech Inc.), 40 units of placental ribonuclease inhibitor (Promega), 1 mM dithiothreitol, 1 μg of linear plasmid template DNA, and 50 units of T7 RNA polymerase (Promega). The reaction was incubated at 37 °C for 45 min, a fresh aliquot of rNTPs and RNA polymerase were added, and the incubation was continued for another 45 min. The reaction was then incubated with 2 units of RQ1 RNase-free DNase (Promega) for 15 min at 37 °C and then extracted with phenol and chloroform and precipitated with ethanol. Approximately 50 ng of RNA was injected into defolliculated stage V-VI *Xenopus* oocytes (19). The injected oocytes were incubated for 4 days at 12 °C in ND-96 buffer containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.5. For transport assays, oocytes were incubated in ND-96 with the appropriate radiolabeled ligand (*e.g.* [^{3}H]2-DOG at $0.8–4 \times 10^4$ μCi $mmol^{-1}$) for 1 h at ambient temperature, removed from the ligand and rapidly rinsed three times in fresh ND-96, dissolved in 1 ml of 1% Triton X-100, mixed with 10 ml of Bio-Safe II fluor, and counted on a liquid scintillation counter.

Estimation of K_m Values from Substrate Saturation Curves—K_m values were determined by fitting substrate saturation data by least squares to the Michaelis-Menten equation ($V = V_{max}S/[K_m + S]$) using the Kaleidagraph program (Synergy Software) employing the Levenberg-Marquardt algorithm. In addition, double reciprocal plots were constructed and fitted by least squares linear regression using the Cricket Graph III program (Computer Associates).

RESULTS

Cloning of Entire Protein Coding Region of iso-1—In a previous report (13) we identified iso-1 as a gene that is distinct from iso-2 by subcloning and sequencing a portion of iso-1 which encodes amino acids 1 through 437. To confirm that the only differences between iso-1 and iso-2 are in the amino-terminal hydrophilic domain and to obtain functional expression of iso-1, it was first necessary to clone and sequence the complete 651-amino acid coding region of this isoform. To obtain such a clone we designed one oligonucleotide primer which contained sequence surrounding the amino terminus of iso-1 and a second primer which contained sequence immediately downstream from the carboxyl terminus of iso-2 (and presumably iso-1 as well) and used these primers in the polymerase chain reaction (20) to amplify a 2-kilobase pair segment of genomic DNA from *L. enriettii*. This polymerase chain reaction amplified segment was subcloned and sequenced. The predicted amino acid sequence of this subclone (data not shown) revealed that iso-1 is indeed identical to iso-2 over the entire region beginning at amino acid 133 of iso-1 and continuing through carboxyl-terminal amino acid number 651. Hence the iso-1 and iso-2 proteins contain completely different amino-terminal domains (amino acids 1–132 of iso-1 and 1–48 of iso-2), but they are identical in sequence beginning with the first putative transmembrane segment and continuing to the end of the protein coding region (Fig. 1).

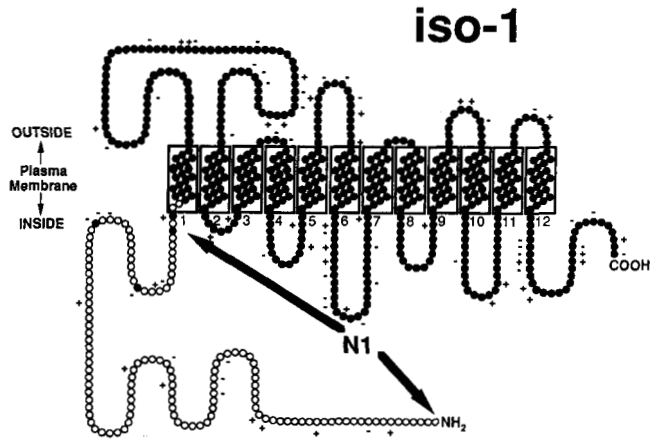

FIG. 1. **Schematic representation of iso-1 indicating the unique NH_2-terminal domain (amino acids 1–132, delineated by the *arrows* marked *N1*) which differs from iso-2.** *Black circles* designate amino acids that are identical between iso-1 and iso-2, whereas *open circles* are amino acids which are unique to iso-1. Putative transmembrane domains (inside open rectangles) have been predicted using the Eisenberg algorithm (35), *plus* signs are lysine or arginine residues, *minus* signs are aspartate or glutamate residues, and *diamonds* are charged residues within transmembrane domains. This figure has been modified from a previous depiction of iso-2 in Fig. 4 of Ref. 36.

Functional Expression of iso-1 and iso-2 in Xenopus Oocytes—In order to express each Pro-1 isoform to determine its function and substrate specificity, the protein coding regions of iso-1 and iso-2 were subcloned into the *Xenopus* oocyte expression vector L2–5 (18). RNA was prepared from each clone by transcription with T7 RNA polymerase and microinjected into *Xenopus* oocytes. Four days after injection these oocytes, as well as controls microinjected with water, were assayed for transport of the glucose analogue [^{3}H]2-deoxy-D-glucose (2-DOG). This analogue has been utilized routinely in studies of glucose transport in *Leishmania* because it can be phosphorylated by hexokinase but cannot be further metabolized by the parasites (6). Fig. 2 (*A* and *B*) shows that oocytes expressing each isoform transported this ligand at a linear rate for at least 3 h and accumulated the substrate to a 50-fold higher level than the control oocytes at the 3-h time point. Furthermore, this transport activity could be completely abrogated by competition with unlabeled D-glucose but not L-glucose (Fig. 3). These results prove conclusively that both the iso-1 and iso-2 forms of the Pro-1 protein are bona fide glucose transporters.

Previous studies on glucose transport by *L. donovani* (7) have revealed that transport of this sugar can be strongly inhibited by other hexoses such as fructose and mannose and suggest that these sugars are also likely to be substrates for the parasite glucose transporter. The ability to functionally express cloned *Leishmania* glucose transporters now allows us to directly test this hypothesis and to eliminate the alternative interpretation that these inhibitors might bind to the active site of the transporter but fail to be transported. In competition experiments (Fig. 3) we have confirmed that fructose and mannose can completely inhibit transport of [^{3}H]2-DOG in promastigotes of *L. enriettii*, whereas other sugars such as L-glucose or D-ribose have little or no effect on transport of this ligand even when included at a 200-fold higher concentration than the ra-

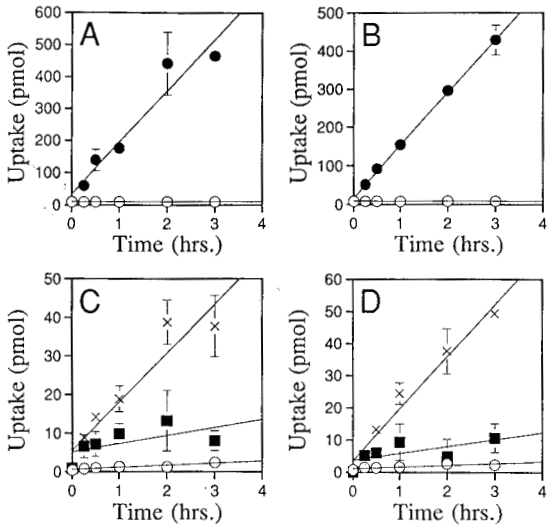

FIG. 2. **Time courses for transport of radiolabeled sugars in oocytes injected with iso-1 (*A* and *C*) or iso-2 (*B* and *D*) RNA.** Injected oocytes were incubated with 50 μM [^{3}H]2-DOG (●), 50 μM [^{14}C]fructose (×), or 50 μM [^{3}H]mannose (■). For each time point, uptake into three oocytes was measured and averaged; *error bars* represent standard deviations of these three values. *Open circles* (○) represent control oocytes injected with water instead of RNA. The uninjected controls in *parts C* and *D* were measured in the presence of 50 μM [^{14}C]fructose.

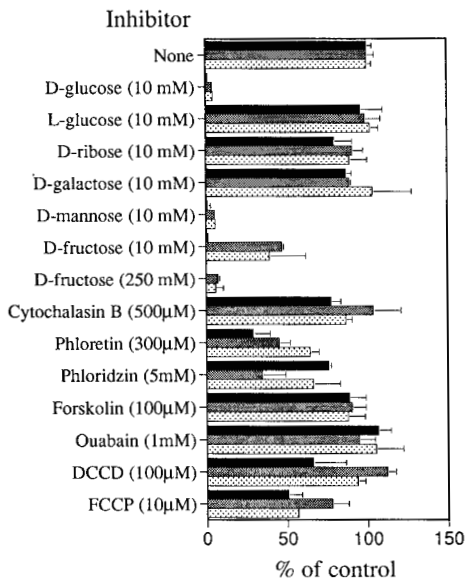

FIG. 3. **Inhibition of [^{3}H]2-DOG transport by various reagents in promastigotes of *L. enriettii* (*solid bars*), and in oocytes injected with iso-1 (*cross-hatched bars*) or iso-2 (*dotted bars*) RNA.** For each measurement, the concentration of 2-DOG was 50 μM and the concentration of inhibitor was as indicated in the figure. Assays in *L. enriettii* were performed for 1 min, and those in oocytes for 1 h. Each *bar* represents the average of three independent measurements; values were averaged, and *error bars* indicate standard deviations.

diolabeled ligand. Fig. 2 (*C* and *D*) demonstrates that oocytes injected with iso-1 or iso-2 RNA are able to transport D-[^{14}C]fructose and D-[^{3}H]mannose and accumulate these sugars to significantly higher levels than control oocytes. However, transport of mannose is relatively weak compared to 2-DOG or fructose, suggesting that mannose is a very poor substrate for both transporters. These results confirm that these *Leishmania* glucose transporters have a relatively broad substrate specificity and are able to recognize ligands that are not substrates for mammalian glucose transporters such as GLUT1.

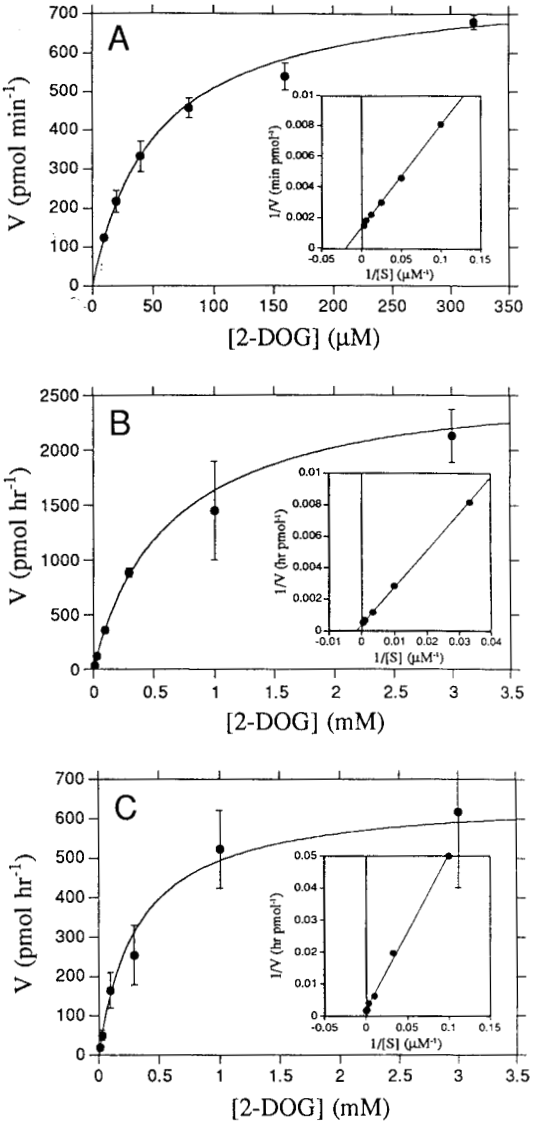

FIG. 4. **Substrate saturation curves for [^{3}H]2-DOG measured in promastigotes of *L. enriettii* (*A*), in oocytes injected with iso-1 RNA (*B*), and in oocytes injected with iso-2 RNA (*C*).** For each substrate concentration, at least three independent samples were measured and the calculated velocities were averaged; *error bars* represent standard deviations of these values. Velocity values in *A* are per 5×10^7 parasites and in *B* and *C* are per oocyte. The *insets* display double reciprocal plots of the data in the saturation curves. The duration of transport assays was 1 min in promastigotes and 1 h in oocytes.

Estimation of Apparent K_m Values for iso-1 and iso-2—Functional differences between glucose transporter isoforms can be reflected in distinct kinetic parameters. Thus isoforms of the human facilitated glucose transporters often have significantly different K_m values for glucose, correlated with their particular physiological roles and with the range of substrate concentration over which they must function optimally (21). Hence, it is important to determine whether the half-saturating substrate levels for iso-1 and iso-2 are different and might reflect distinct functional roles for each isoform.

Fig. 4 shows representative substrate saturation curves and double reciprocal plots (*insets*) for [^{3}H]2-DOG measured in promastigotes of *L. enriettii* (Fig. 4*A*) and in oocytes expressing either iso-1 (Fig. 4*B*) or iso-2 (Fig. 4*C*). To estimate K_m values for each system, at least three independent substrate saturation curves were measured, and these data were fit to the Michaelis-Menten equation as described under "Experimental Procedures." Promastigotes of *L. enriettii* show reproducible K_m

values of 53 ± 0.6 μM (n = 3), in reasonable agreement with the K_m of 24.4 μM measured previously in promastigotes of *L. donovani* using similar methods (6). The kinetic values determined from saturation curves in injected oocytes are less reproducible but indicate a K_m for iso-1 of 647 ± 257 μM (n = 3) and a K_m for iso-2 of 285 ± 55 μM (n = 4). Hence, the K_m for iso-1 appears to be slightly higher than that for iso-2 but is not dramatically different given the scatter inherent in these measurements. However, the K_m values measured in oocytes are significantly higher than those determined in the parasites. To determine whether oligomerization of iso-1 and iso-2 could produce a transporter with higher substrate affinity, we co-expressed both proteins within single oocytes. The K_m values obtained in these co-expression experiments (456 ± 222 μM for n = 2) were not significantly different from those measured in oocytes expressing each isoform alone.

Inhibition of 2-Deoxy-D-glucose Transport by Other Substrates and Inhibitors—In previous experiments (7) the sensitivity of glucose transport in whole promastigotes of *L. donovani* to inhibition by various sugars and organic reagents has been studied. In particular, D-glucose, D-fructose, D-mannose, *N*-acetyl-D-glucosamine, D-glucosamine, and D-galactose inhibited transport of 2-DOG with decreasing order of affinity, suggesting that some or all of these sugars are substrates for promastigote glucose transporters. Cytochalasin B, which is a potent inhibitor of mammalian facilitated glucose transporters (22), inhibits glucose transport in promastigotes but does so with an IC_{50} of 55 μM (23), about 100-fold higher than that observed using mammalian systems. DCCD, an inhibitor of proton ATPases, and FCCP, an ionophore that uncouples proton gradients (23), significantly inhibit glucose transport in *L. donovani* promastigotes (8), consistent with a possible role for proton gradients in energizing glucose transport in *Leishmania* species.

We have tested the ability of these reagents to inhibit transport of 2-DOG in promastigotes of *L. enriettii* and in oocytes microinjected with iso-1 or iso-2 RNA. Fig. 3 shows that D-glucose, D-fructose, and D-mannose inhibit transport of 2-DOG in parasites and in oocytes injected with RNA for each isoform, whereas L-glucose, D-ribose, and D-galactose do not significantly inhibit such transport at these concentrations. Hence, in general the inhibition patterns of iso-1 and iso-2 are very similar to each other and to that of glucose transport in promastigotes of *L. enriettii*. One exception occurs in relation to fructose inhibition of 2-DOG transport: 10 mM fructose will strongly inhibit transport of 50 μM 2-DOG in promastigotes but only inhibits such transport by about 50% in oocytes injected with either iso-1 or iso-2. However, 250 mM fructose will strongly inhibit 2-DOG transport in microinjected oocytes. One possible explanation for this discrepancy is that the K_i for fructose is higher in oocytes than it is in the parasites, thus requiring a higher concentration of fructose to inhibit transport of 2-DOG.

Fig. 3 also shows inhibition by various organic reagents of 2-DOG transport in *L. enriettii* promastigotes and in oocytes injected with iso-1 or iso-2 RNA. Cytochalasin B, phloretin (24) (an inhibitor of mammalian facilitated glucose transporters), phloridzin (25) (an inhibitor of mammalian Na^+-dependent glucose transporters), and FCCP achieve moderate inhibition of transport in promastigotes and oocytes injected with RNA for both isoforms, whereas DCCD inhibits somewhat in promastigotes but not in oocytes. Forskolin, a potent inhibitor of mammalian facilitated glucose transporters (26), and ouabain, an inhibitor of Na^+/K^+-ATPase and hence indirectly of Na^+-dependent symporters, do not inhibit transport in any of these systems. Hence, the pharmacology of each isoform is similar to the pharmacology of glucose transport in promastigotes. Modest differences between our results in *L. enriettii* and the previously reported results in *L. donovani* are that: (i) cytochalasin B is a less potent inhibitor of glucose transport in *L. enriettii*, producing about 25% inhibition at a 500 μM concentration compared to 50% inhibition at 55 μM drug in *L. donovani*; (ii) DCCD and FCCP are not as effective inhibitors in the *L. enriettii* cells as they are in *L. donovani*, where similar concentrations inhibited transport by approximately 70 and 85%, respectively; and (iii) phloridzin (5 mM) is a more effective inhibitor in *L. enriettii* (about 25%) than it is in *L. donovani* (about 10%).

DISCUSSION

During their life cycle *Leishmania* parasites must adapt to the markedly different environments of the sandfly gut and the macrophage lysosome. Each of these two habitats presents a different pH, temperature, and range of nutrients available for metabolism. While the promastigotes are exposed to sugars such as glucose when they are in the sandfly, initially from the blood meal and later from the plant sap meals that constitute the sandfly diet (27), the amastigotes live in a relatively sugar poor intracellular compartment (6). Furthermore, promastigotes utilize glucose metabolically at a rather robust rate, whereas amastigotes are much less dependent upon glycolysis for a source of energy and rely more heavily upon the oxidation of fatty acids (3). Hence, these parasites might be expected to down-regulate glucose transport upon transformation of promastigotes to amastigotes or possibly to induce expression of a different glucose transport system more ideally suited for life within the macrophage lysosome.

In this paper, we have investigated the biological function of the Pro-1 genes which are expressed almost exclusively in the promastigote stage of the life cycle and encode proteins related in sequence and structure to mammalian facilitated glucose transporters (11). The most significant conclusion of this work is that the Pro-1 genes, whose function was previously speculative, do indeed encode proteins that function as glucose transporters. This conclusion has been proven by the ability of both iso-1 and iso-2 to transport the glucose analogue 2-deoxy-D-glucose when each gene is expressed in *Xenopus* oocytes. Furthermore, expression of each gene has allowed us to prove that these proteins do have the previously proposed broad substrate specificity and are able to transport other hexoses such as fructose and mannose. These results parallel those of others who have previously expressed glucose transporters from mammals (28) and from the related Kinetoplastid parasite *Trypanosoma brucei* (29) using the *Xenopus* oocyte system.

The identification of two isoforms of Pro-1 raises the question of what distinct biological function each isoform might perform. The observation that both iso-1 and iso-2 have similar K_m values for 2-DOG and closely related patterns of inhibition by sugars or other organic compounds implies that each isoform has almost identical innate biochemical properties. Hence the biological distinction between these two isoforms either: (i) relies upon some unique properties that we have not been able to measure with the oocyte system, such as kinetic differences that require interactions with other parasite proteins not present in the oocytes; or (ii) is conferred by a non-kinetic property of the proteins. One such non-kinetic distinction between these isoforms might involve unique subcellular localization of each isoform. If iso-1 and iso-2 are differentially localized, then the unique NH_2-terminal domain must contain a localization signal in at least one of the isoforms. A precedent for such a dominant localization signal within the NH_2 terminus of a glucose transporter has been suggested for the insulin-regulated mammalian glucose transporter GLUT4 (30, 31). The NH_2 terminus of this protein is required to target GLUT4 to cytoplasmic vesicles, and deletion or alteration of the first 7 amino acids of

this domain causes retargeting of the transporter to the plasma membrane. To address the question of differential targeting, we are currently attempting to localize each isoform of Pro-1 by immunocytochemical methods.

Although the kinetic properties of iso-1 and iso-2 measured in microinjected oocytes are very similar to each other, the K_m values are significantly higher than those measured for promastigotes of *L. enriettii*. There are several potential explanations for these differences. The physiological microenvironment of the oocytes may differ in some significant way from that of the parasites, leading to an alteration of the kinetic properties. Alternatively, protein-protein interactions present in the native cellular environment may be absent in the *Xenopus* expression system, which synthesizes only a single parasite protein; these putative interactions might lower the K_m of the transporter in the promastigotes below that which is observed in oocytes.

However, an alternative interpretation of the data is that the K_m values measured in oocytes are accurate representations of the transporter kinetics, and that the K_m measured in intact parasites is artifactually low. Since 2-DOG can be phosphorylated by hexokinase, the uptake measurements represent both transport and phosphorylation. If phosphorylation is the rate-limiting step in total uptake of 2-DOG within the parasites, as reported previously (9), and the K_m for hexokinase is lower than that of the transporter, then the K_m determined from total uptake measurements would be significantly reduced below the true K_m of the transport step. In experiments performed in a chemostat (9), ter Kuile and Opperdoes (9) have measured the K_m for glucose transport in *L. donovani* after correcting for the effect of hexokinase and have obtained values ranging from 0.4 to 0.9 mM; these values are in close agreement with those we have measured in oocytes expressing either iso-1 or iso-2. Furthermore, chromatographic analysis (32) of radiolabeled 2-DOG accumulated within the oocytes (data not shown) reveals that the sugar is all converted to the phosphorylated form, suggesting that the hexokinase step is not rate-limiting in the oocyte experiments. Taken together, these results suggest that the true K_m values for iso-1 and iso-2 are likely to be within the range reported for the oocyte measurements.

Another important question regarding glucose transport in *Leishmania* parasites is whether such transport is active and driven by a proton electrochemical gradient (8) or facilitated and governed by diffusion (9). One potential way to resolve this issue would be to search for a glucose-dependent proton current by voltage-clamping oocytes expressing iso-1 and iso-2. The detection of such a substrate and proton-dependent current would constitute very strong evidence in favor of active transport via proton symport, a mechanism which is believed to apply to sugar transport in several other members of the "facilitated" glucose transporter superfamily such as the arabinose and xylose transporters from *Escherichia coli* (33). We have attempted to measure such currents in oocytes expressing iso-1 or iso-2 in which the level of 2-DOG transport was occurring at a rate of 87 pmol min^{-1} per oocyte (at 2 mM 2-DOG). If such oocytes are co-transporting protons with a stoichiometry of 1:1 compared to glucose, a current of approximately 140 nA should be induced. In several such experiments, we have not been able to detect such a proton current. However, since these are negative results, no conclusion can be drawn as to whether glucose transport is truly facilitated or whether it occurs by some active but non-electrogenic mechanism such as cotransport or antiport.

The ability to functionally express the Pro-1 transporters now opens the route to detailed structure-function studies similar to those currently being pursued in related transport systems such as the *E. coli* lactose permease (34). This advance will allow the combined application of molecular genetic and biochemical tools to the analysis of nutrient transport in these important pathogens. Studies on such developmentally regulated membrane transport proteins should also shed light upon the mechanisms whereby these parasites adapt to and colonize their insect vectors and their vertebrate hosts during the parasite life cycle.

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