Localization and Targeting of the *Leishmania* donovani Hypoxanthine-Guanine Phosphoribosyltransferase to the Glycosome*

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Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) is a key enzyme in the purine salvage pathway of many protozoan parasites. The predicted amino acid sequences of certain HGPRT proteins from parasites of the Trypanosomatidae family reveal a COOH-terminal tripeptide signal that is consistent with the degenerate topogenic signal targeting proteins to the glycosome, a fuel-metabolizing microbody unique to these parasites. To determine definitively the intracellular milieu of HGPRT in these pathogens, polyclonal antiserum to the purified recombinant HGPRT from *Leishmania donovani* was generated in rabbits, and confocal and immunoelectron microscopy were employed to establish that the *L. donovani* HGPRT is localized exclusively to the glycosome. No HGPRT protein was detected in Δhgprt null mutants in which both alleles of the HGPRT locus had been replaced by a drug-resistance cassette. Transfectants of the Δhgprt knockout strain in which a wild-type HGPRT was amplified on an expression plasmid contained augmented amounts of HGPRT, all of which was localized to the glycosome. Δhgprt transfectants containing amplified copies of a mutated HGPRT construct in which the Ser-Lys-Val COOH-terminal targeting signal had been deleted expressed HGPRT throughout the parasite, including subcellular organelles such as the nucleus and flagellum. These data demonstrate that the *L. donovani* HGPRT is compartmentalized exclusively within the glycosome and that the COOH-terminal tripeptide of the protein is necessary to achieve targeting to this organelle.

The genus *Leishmania*, a member of the Trypanosomatidae family, is the causative agent of leishmaniasis, a parasitic disease of considerable import in terms of both mortality and morbidity. All *Leishmania* sp. are digenetic, existing as extracellular, flagellated promastigotes within their sandfly vectors and as intracellular, nonmotile amastigotes within the phagolysosomes of macrophages and other reticuloendothelial cells of the vertebrate host. The drugs used in the treatment of leishmaniasis and other parasitic diseases have been realized empirically and are far from ideal. Toxicity is common, the drugs are potentially mutagenic and/or carcinogenic, regimens are prolonged and usually require multiple drug administration, drug resistance or refractoriness persists as a significant therapeutic obstacle, and the prospects for effective antiparasitic vaccines in the immediate future are bleak (1).

The rational development of new antiparasitic drugs that are selective for the metabolic machinery of the parasite will require therapeutic exploitation of fundamental biochemical differences between the parasite and vertebrate host. Perhaps the most striking metabolic discrepancy between parasites and their human hosts is the pathways by which they synthesize purine nucleotides. Whereas mammalian cells synthesize purine nucleotides from amino acids and one-carbon moieties, all protozoan parasites evaluated to date are incapable of purine nucleotide synthesis *de novo* (2). Thus, these pathogens are auxotrophic for purines and consequently express purine salvage enzymes that enable them to access host purines.

Metabolic, biochemical, and genetic studies have revealed that *Leishmania donovani* promastigotes funnel a variety of exogenous purines into hypoxanthine (3, 4), intimating that the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) plays a central role in this purine acquisition process. However, the viability and normal growth rate of Δhgprt null mutants of *L. donovani* that have been created by targeted gene replacement have clearly demonstrated that HGPRT does not play an indispensable role in purine salvage by the promastigote form of the parasite (5, 6). Whether a functional HGPRT activity is crucial to the infective form of the parasite is unknown. The trypanosomatid HGPRT also initiates the intracellular metabolism of allopurinol (HPP, 4-hydroxypyrazolo[3,4]pyrimidine), an effective antileishmanial and antitrypanosomal agent (7, 8) that is nontoxic to humans and widely used in the treatment of hyperuricemia and gout (9). Indeed, HPP has demonstrated significant therapeutic efficacy in patients with either cutaneous leishmaniasis (10) or chronic Chagas disease (11).

Subcellular fractionation experiments have suggested that the HGPRT activities in *Leishmania mexicana* (12), *Trypanosoma brucei* (13), and *Trypanosoma cruzi* (14) are localized to the glycosome, a fuel metabolizing microbody that is unique to parasites of the Trypanosomatidae family (15, 16). A glycosomal milieu for HGPRT in the Trypanosomatidae is supported by the fact that the COOH-terminal tripeptides of the HGPRTs from *L. donovani* (17), *T. cruzi* (18), and *Crithidia fasciculata*...
are consistent with the acceptable degeneracy for the well characterized COOH-terminal targeting signal for glycosomal localization (20, 21). As definitive information on the subcellular location of therapeutically pertinent enzymes is critical for drug targeting, we have established by confocal and immunoelectron microscopy that the HGPRT protein from *L. donovani*, unlike the mammalian HGPRT, is localized exclusively to the glycosome. Furthermore, we have demonstrated genetically that the COOH-terminal tripeptide is required for this glycosomal compartmentation, as Δhgprt mutants transfected with an hgprt construct truncated at the COOH terminus express HGPRT protein throughout the parasite.

**EXPERIMENTAL PROCEDURES**

*Parasites—* *L. donovani* promastigotes were grown at room temperature in Dulbecco’s modified Eagle’s-Leishmania medium with xantine as the purine source (22). Cells employed in the biochemical or immunolocalization experiments were routinely harvested in exponential growth at a density of ~5–6 × 10^6 cells/ml. The wild-type DI700 strain originates from the 1S Sudanese clone of *L. donovani* and is designated DI700:H^+/+ for the purposes of the genetic manipulations described in these studies, where H denotes the HGPRT locus and + specifies the wild-type allele. Mutant parasites containing functional copies of the neomycin or hygromycin phosphotransferase genes were routinely maintained in Dulbecco’s modified Eagle’s-Leishmania medium supplemented with 50 μg/ml Geneticin (G418) (Life Technologies, Inc.) or 400 μg/ml hygromycin, respectively.

*Construction of Leishmanial Expression Vectors and Transfection into *L. donovani*—*The* *L. donovani* HGPRT and flanking regions were isolated as described (5, 17). Vectors pX63-HYG and pSNBR (containing the neomycin phosphotransferase gene) have been described (23, 24) and were provided by Dr. Stephen M. Beverley of Washington University. The construction of the Δhgprt null mutant, DI700:H^+/n has also been described previously (5), where the n superscript designates an allele in which the neomycin phosphotransferase marker has been inserted into the HGPRT locus. The DI700:H^+/n strain was generated by replacing the first wild-type HGPRT allele with a targeting construct encompassing the neomycin phosphotransferase marker followed by negative selection for loss of heterozygosity to generate the Δhgprt knockout (5). The DI700:H^+/n strain was employed as a recipient for transfection of functional episomal HGPRT constructs.

To create HGPRT overexpressors, wild-type and mutant HGPRT constructs were inserted into leishmanial expression vectors and amplified by selection in hygromycin. The wild-type HGPRT was amplified from the 3.5-kilobase EcoRI HGPRT fragment initially described by Allen *et al.* (17) using the polymerase chain reaction (PCR) and a Coy Instruments (Ann Arbor, MI) thermocycler. Standard conditions for amplification of plasmid DNA were employed: 15 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 0.5 min, and extension at 72 °C for 0.5 min. The sense primer, 5'-GAAGATCTTATAGCTCGTCACCG-3', was constructed to the first 18 nucleotides of the HGPRT gene coding region and was preceded by a 9-nucleotide leader encompassing an XmaI restriction site (underlined). The antisense primer, 5'-TCCCCGGGATGAGCAAC-TGGGCGGAGCAGCAGCCACCC-3', was constructed to the first 18 nucleotides immediately downstream of the HGPRT gene coding region including the stop codon (bold lettering) and contained an 8-nucleotide leader with a BglII restriction site (underlined). The 668-bp PCR product was digested with appropriate restriction enzymes and ligated into the XmaI-BglII sites of the leishma-
FIG. 3. Transmission electron micrographs of ultrathin cryosections of wild-type *L. donovani* immunolabeled with anti-HGPRT and anti-GAPDH antibodies. Wild-type DI700: H^{+/+} *L. donovani* were fixed in either 4% paraformaldehyde and 0.1% glutaraldehyde (A) or 8% paraformaldehyde (B) and immunolabeled with anti-HGPRT antisera followed by goat anti-rabbit immunoglobulin conjugated to gold (10-nm probe). As a control, parasites fixed in 8% paraformaldehyde were immunolabeled with anti-GAPDH antibodies followed by immunogold probe (C). DI700: H^{+/+} cells fixed in 4% paraformaldehyde and 0.1% glutaraldehyde were double-labeled with anti-HGPRT (5-nm gold particles indicated with
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A mutant HGPRT lacking the three COOH-terminal amino acids was also amplified from the same template using identical PCR conditions and sense primer. The antisense primer, 5'-GGATCTAGAATCTCCGGCTTCTCGTAGTACTC-3', was constructed to amino acids 202-208 of the wild-type HGPRT and was preceded with a 5'-GTCCGAGATCT restriction site (underlined) and an in-frame stop codon (bold). After cleavage with XmaI and XbaI, the 645-bp PCR fragment was ligated into the corresponding sites of the pSNBR-HYG vector.

pSNBR-HYG was generated by replacing the neomycin phosphotransferase gene (neo) from pSNBR-NEO with the hygromycin phosphotransferase gene (hgy) from pX63-HGPRT (Promega, Madison, WI). The 3,468-bp KpnI-HindIII fragment encompassing neo was inserted into a modified pBluescript plasmid in which the SpeI and SacI sites had been destroyed. The neo marker was then replaced by ligating the 1,843-bp SpeI-SacI fragment of pX63-HGY into the resulting vector from which the neo marker had been excised with SpeI and SacI. Finally, the KpnI-HindIII fragment encompassing hgy was ligated to the 2,927-bp KpnI-HindIII fragment from pSNBR that contained the prokaryotic origin of replication and the ampicillin resistance gene.

The wild-type and COOH-terminal deletion mutant HGPRT constructs were designated pX63-HGY-HGPRT and pSNBR-HYG-hgprt (209-211), respectively. The COOH-terminal deletion within the pSNBR-HYG-hgprt(209-211) construct was confirmed by dideoxy-nucleotide sequencing using the Sequenase Version 2.0 kit (United States Biochemical Corp.). pX63-HGY-HGPRT and pSNBR-HYG-hgprt(209-211) were transfected into the D700-H7/n HGPRT parasites using standard electroporation conditions for Leishmania parasites (5, 6, 23) and selected for resistance to increasing concentrations of hygromycin up to a final concentration of 400 μg/ml. D700-H7/n cells transfected with pX63-HGY-HGPRT and pSNBR-HYG-hgprt(209-211) were designated D700-H7/n (pX63-HGY-HGPRT) and D700-H7/n (pSNBR-HYG-hgprt (209-211)), respectively. For the purposes of figure representation, D700-H7/n (pX63-HGY-HGPRT) and D700-H7/n (pSNBR-HGY-hgprt(209-211)) were labeled W2 and ASK, respectively.

Expression of Wild-type and Mutant HGPRT Constructs in E. coli—The construction of the wild-type HGPRT pBAce expression vector (17) and the wild-type HGPRT-pSelect mutagenesis expression vector (24) have been reported. To generate an HGPRT expression vector in which the COOH-terminal tripeptide was deleted, the HGPRT within pSelect (Promega, Madison, WI) was altered by introduction of three stop codons (boldface in primer below) at positions 209-211 of the protein. The mutagenic primer was 5'-CTCAAGAAGGATCTACATGGCGAGGAGTGTAGTGAATCTCCGGCTTCTCGTAGTACTC-3' and contained a PstI site (underlined) at the 3' end. The mutated pXel-PstI HGPRT fragment from pSelect was then ligated into the appropriate sites in pBAce and transformed into S6060 (Aprom-prl-fac, thid, hpl) E. coli, a strain lacking the bacterial hypoxanthine and xanthine-guanine phosphoribosyltransferase enzymes (26). The COOH-terminal truncation was verified by nucleotide sequencing.

Enzyme Purification and Assay—Recombinant wild-type and mutant HGPRT were purified to homogeneity by affinity chromatography as reported (17, 27). Activity of recombinant enzyme was assayed spectrophotometrically as described (25). HGPRT activity in sonicated extracts of exponentially growing parasites (~1 x 10^7 parasites/ml) that had been extensively washed with phosphate-buffered saline (PBS) was assessed by a previously described radiometric protocol (27). Protein was measured by the method of Bradford (28).

Antibodies—Polyclonal antisera to recombinant HGPRT was generated as described (5). The antisera was purified by chromatography over a protein A-Sepharose column (Sigma) according to the brochure of the supplier. Rabbit antisera raised to the glycosomal glycerolaldehyde-3-phosphate dehydrogenase (GAPDH) protein of L. mexicana were generously provided by Dr. P. Michels and F. Oppendoes of the International Institute of Cellular and Molecular Pathology in Den based, Belgium.

Immunoblotting—Wild-type, mutant, and transfectant parasites were harvested by centrifugation, washed with PBS, resuspended in sample buffer, and heated to 100 °C for 3 min. Lysates from 10^7 cells in a volume of 20 µl were fractionated on a 15% SDS-polyacrylamide gel (29) and electrotransferred to nitrocellulose membranes. The nitrocellulose filters were incubated with anti-HGPRT antisera at a 1:10,000 dilution using a blocking solution of 5% dry milk, and the blots were developed using the ECL chemiluminescence system (Amersham Corp.) and goat anti-rabbit IgG coupled to horse-radish peroxidase (Boehringer Mannheim).

Immunofluorescence Microscopy—Exponentially growing L. donovani at concentrations (~5 x 10^7 cells/ml) were harvested by centrifugation and washed several times in PBS, incubated for 30 min in fixative consisting of 50% acetone-hepes buffer, pH 7.2, 4% paraformaldehyde, and 0.1% glutaraldehyde, and attached to coverslips coated with poly-L-lysine. Cells were then permeabilized for 30 min at ambient temperature in PBS containing 1% Triton X-100 and 5% goat serum. Anti-HGPRT antibody at a dilution of 1:200 was added to the permeabilized cells and incubated either for 4 h at 25 °C or for 16 h at 4 °C. Coverslips were rinsed six times with PBS, incubated with goat anti-rabbit IgG secondary antibody conjugated to Texas Red fluorophore (Molecular Probes, Eugene, OR) at a 1:500 dilution, and incubated overnight at 4 °C in the absence of light. After six more washes in PBS, a fluorescein isothiocyanate dyed, 3,3-dihexyloxyacarbocyanine iodide (Molecular Probes), was added to a concentration of 1 μg/ml, and the coverslips were washed immediately with PBS. Coverslips were mounted on slides in 50 mM Tris, pH 8.0, 90% glycerol, and 20 mg/ml n-propyl gallate and examined with a confocal laser scanning microscope as described (31).

Immunoelectron Microscopy—Cells were harvested and fixed either with 4% paraformaldehyde in 50 mM acetone-hepes buffer, pH 7.2, or as described (27). Fixed cells were incubated overnight with the recombinant HGPRT antibody, then washed, and incubated with goat anti-rabbit IgG secondary antibody conjugated to Texas Red fluorophore. Fixed and permeabilized cells were rinsed three times with PBS, incubated with goat anti-rabbit IgG secondary antibody conjugated to Texas Red fluorophore, and washed three times with 100 μl hepes buffer, pH 7.2, then infused with polyvinylpyrrolidone and sucrose (32) for ultrathin cryosectioning. Double labeling using two different sizes of gold probes (Amersham Corp.) was performed as described (33). 1% glutaraldehyde in PBS was used for the blocking step between antibodies. Control incubations included labeling cyrossections with either 1) anti-HGPRT antibodies competed with purified recombinant HGPRT, 2) no primary antibodies, or 3) irrelevant antibodies. Immunolabeled sections were embedded in 25% centoise methyl cellulose and uranyl acetate (34) and viewed with a Phillips 301 transmission electron microscope (Philips Electronics, Mahwah, NJ).

RESULTS

Antibody Characterization—To determine the location of the HGPRT in L. donovani, antibodies directed against epitopes on the enzyme were generated in rabbits. These antibodies recognized only a single protein in wild-type parasite lysates that migrated with a molecular mass approximate to that predicted from the translated HGPRT protein coding region (Fig. 1). This band was established to be HGPRT genetically as this antisera did not recognize any protein in lysates of the Δhgprt D700-H7/n strain (Fig. 1).

HGPRT Is Targeted to L. donovani Glycosomes—The location of the HGPRT enzyme in wild-type L. donovani was initially assessed by confocal microscopy. Immunofluorescence analysis of HGPRT stained with Texas Red-conjugated secondary antibody revealed a punctate pattern in D700-H7/n cells not observed with D700-H7/n parasites (Fig. 2, A and B, respectively). Immunoelectron microscopy was used to confirm glycososomal targeting of the enzyme (Fig. 3). Gold particle labeling for HGPRT was sequestered within the glycosomes in wild-type parasites, and no other subcellular compartments were labeled to a significant degree (Fig. 3, A and B). This localization pattern paralleled that obtained with antibodies to L. mexicana GAPDH (Fig. 3C), a glycolytic enzyme whose glycosomal location in L. donovani has been documented (35, 36). Furthermore, the L. donovani HGPRT colocalized with the GAPDH (Fig. 3D). No immunogold labeling was observed for either D700-H7/n cells incubated with excess recombinant HGPRT (Fig. 3E) or for D700-H7/n parasites (Fig. 4, A and B). These data demonstrate clearly that the L. donovani HGPRT is compartmentalized to the glycosome.

arrowheads) and with anti-GAPDH (10-nm gold particles) as indicated in panel D. Inset within panel D shows a glycosome from a second parasite double labeled in an identical fashion. A control cryosection immunolabeled with anti-HGPRT antisera was competed with excess recombinant HGPRT prior to incubation with the tissue section (E). A, 4 x 40,000; B, 3 x 38,000; C, 3 x 38,000; D, 6 x 62,000 (inset, 6 x 62,000); E, 5 x 50,000, g, glycosome; n, nucleus; v, vacuole; fp, flagellar pocket.
The COOH-terminal Ser-Lys-Val Tripeptide Is Necessary for Glycosomal Targeting—To validate whether the COOH-terminal tripeptide is required for targeting HGPRT to the glycosome, DI700:H\(^{+/+}\) parasites were transformed with expression plasmids containing either a wild-type copy of HGPRT (pX63-HYG-HGPRT) or a mutated construct lacking the codons for the SKV COOH-terminal tripeptide (pSNBR-HYG-hgprt(D209–211)). Both the DI700:H\(^{+/+}\)[pX63-HYG-HGPRT] and DI700:H\(^{+/+}\)[pSNBR-HYG-hgprt(D209–211)] transfectants expressed ~10–15-fold greater quantities of HGPRT activity (Fig. 5) and protein (Fig. 1) than DI700:H\(^{+/+}\) parasites (Fig. 5).

Deletion of the Ser-Lys-Val tripeptide did not appear to grossly alter the catalytic properties of the enzyme. Apparent \(K_m\) and \(k_{cat}\) values calculated for the mutant enzyme by Hanes analysis were 4.8 \(\mu\)M and 6.2 \(s^{-1}\) for hypoxanthine and 11.6 \(\mu\)M and 15.8 \(s^{-1}\) for guanine. These values are comparable with the \(K_m\) and \(k_{cat}\) values of 6.4 \(\mu\)M and 5.7 \(s^{-1}\) for hypoxanthine and 9.9 \(\mu\)M and 12.1 \(s^{-1}\) for guanine that were reported previously for the wild-type \(L.\) donovani HGPRT (17).

Immunofluorescence analysis of DI700:H\(^{+/+}\)[pX63-HYG-HGPRT] cells revealed amplified HGPRT signal in a punctate pattern, whereas HGPRT appeared to be dispersed in the...
The role of the Ser-Lys-Val tripeptide in glycosome targeting was then established by immunoelectron microscopy. Whereas labeling with immunogold was restricted to the glycosomes in DI700:H<sup>n</sup>/<sup>n</sup>[pX63-<span>H</span><sup>G</sup>-<span>HGPRT</span>] transfectants, immunogold particles were ubiquitously distributed throughout DI700:H<sup>n</sup>/<sup>n</sup>[pSNBR-<span>HYG-hgprt</span>(<span>D</span><sub>209–211</sub>)] parasites (Fig. 4, C-F). To ensure that the disseminated distribution of HGPRT in DI700:H<sup>n</sup>/<sup>n</sup>[pSNBR-<span>HYG-hgprt</span>(<span>D</span><sub>209–211</sub>)] cells could not be attributed to glycosome loss or leakage, the location of GAPDH was ascertained in all cell lines by immunofluorescence. Confocal immunofluorescence with antibodies directed against the glycolytic enzyme revealed similar punctate patterns of staining in DI700:H<sup>1</sup>/<sup>1</sup>, DI700:H<sup>n</sup>/<sup>n</sup>, DI700:H<sup>n</sup>/<sup>n</sup>[pX63-<span>H</span><sup>G</sup>-<span>HGPRT</span>], and DI700:H<sup>n</sup>/<sup>n</sup>[pSNBR-<span>HYG-hgprt</span>(<span>D</span><sub>209–211</sub>)] cells (Fig. 6, A-D).

**DISCUSSION**

The HGPRT enzyme of *L. donovani* has been definitively localized to the parasite glycosome by indirect immunofluorescence and immunoelectron microscopy. Specificity of antibodies directed against HGPRT epitopes used for the immunocytochemistry was established by 1) immunoblotting of whole cell lysates, 2) the ability of excess purified recombinant enzyme to block immunogold labeling of the parasites, and 3) the failure of the anti-HGPRT antisera to recognize any protein in Δ<sup>hgprt</sup> parasites. A glycosomal milieu for this central enzyme in purine salvage in trypanosomatids was initially conjectured on the basis of crude subcellular fractionation experiments in which HGPRT activity was largely associated with the glycosomal fraction of *L. mexicana* (12), *T. brucei* (13), and *T. cruzi* (14). Moreover, trypanosomatid HGPRT coding sequences predicted COOH-terminal tripeptides compatible with the well-established COOH-terminal signal that is recognized by the glycosome import machinery (20, 21). Of these three amino acids, the first is usually a small uncharged amino acid, the second an amino acid containing a hydrogen bond-forming group, and the third is hydrophobic in nature (20, 21). The *L. donovani*, *T. cruzi*, and *C. fasciculata* HGPRTs terminate in...
Ser-Lys-Val (17), Ser-Lys-Tyr (18), and Ser-Lys-Leu (19), respectively, and are all within the acceptable degeneracy for this targeting signal. The *T. brucei* HGPRT ends with an Ala-Lys-Arg (37) and would not be predicted from mutational studies of targeting signals on firefly luciferase (21) and chloroamphenicol acetyltransferase-phosphoglycerate kinase hybrids (20) to be a glycosomal import signal.

Mutational analysis has established that the Ser-Lys-Val of the *L. donovani* HGPRT protein is required for glycosome compartmentalization. Transfection of the pSNBR-HYG-hgprt(D209–211) construct in which these three amino acids have been deleted into a Δhgprt background results in a ubiquitous distribution of the protein throughout the parasite, including the nucleus, flagellum, and plasma membrane, as well as glycosomes. This mislocalization of mutated protein cannot be attributed to plasmid expression of *HGPRT*, as a wild-type episomal construct directed the expression of correctly targeted protein, or to major structural abnormalities, as the hgprt(D209–211) protein was kinetically comparable with the wild-type enzyme. Although, the COOH-terminal tripeptide is necessary for glycosome compartmentation, and whether it contains all the necessary information for targeting HGPRT to the glycosome is unknown. However, Ser-Lys-Val addition to the COOH terminus does mediate glycosome entry of firefly luciferase in *T. brucei* (21).

The reason for the glycosomal compartmentalization of HGPRT in *L. donovani* and related parasites remains unclear, as the enzyme is cytosolic in mammalian cell systems. However, glycosomes accommodate a variety of fuel metabolizing enzymes that perform essential nutritional functions for the parasite (15, 16), and the auxotrophy of these parasites for purines (2) dictates that purine salvage is nutritionally essential for these organisms. Therefore, the perspective that glycosomes are fuel metabolizing organelles can perhaps be expanded to include other nutritional pathways. The location of other purine salvage and interconversion enzymes in trypanosomatids has not been conclusively determined. Fractionation of *L. mexicana* extracts by isopycnic centrifugation indicated that the purine salvage enzymes, IMP dehydrogenase, the enzyme that converts the IMP product of HGPRT to XMP, and xanthine phosphoribosyltransferase, cosedimented with glycosome and particulate fractions, respectively, whereas adenine phosphoribosyltransferase, nucleosidases, and adenine deaminase were all cytosolic (38). Interestingly, the COOH-terminal tripeptides of IMP dehydrogenase (39) and xanthine phosphoribosyltransferase (Ala-Lys-Leu, data not reported) from *L. donovani* are both plausible glycosomal import signals. The comparable sequence on adenine phosphoribosyltransferase, His-Pro-His, is an unlikely candidate to mediate glycosome entry (40). One credible hypothesis for the glycosomal compartmentation of HGPRT is to provide optimal access to substrate. However, purine bases must obligatorily be acquired from the

![Fig. 5. HGPRT activity assays in *L. donovani* extracts.](image)
extracellular environment, whereas PRPP substrate is generated from ribose-5-phosphate and ATP by the catalytic action of PRPP synthetase. Although the location of the parasite PRPP synthetase is not known, the enzyme terminates in an Arg-Asp-Ser sequence, a dubious targeting signal for glycosome entry. Despite the lack of a clear rationale for the atypical organellar synthetase, a dubious targeting signal for glycosome entry.

Ser sequence, a dubious targeting signal for glycosome entry. Although the location of the parasite PRPP synthetase is not known, the enzyme terminates in an Arg-Asp-Ser sequence, a dubious targeting signal for glycosome entry.

Despite the lack of a clear rationale for the atypical organellar synthetase, a dubious targeting signal for glycosome entry.

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