

## Purification and characterization of hexokinase from *Leishmania mexicana*

Miguel A. Pabón · Ana J. Cáceres · Melisa Gualdrón ·  
Wilfredo Quiñones · Luisana Avilán ·  
Juan L. Concepción

Received: 7 August 2006 / Accepted: 26 September 2006 / Published online: 24 October 2006  
© Springer-Verlag 2006

**Abstract** Hexokinase from *Leishmania mexicana* was purified to homogeneity from a glycosome-enriched fraction obtained after a differential centrifugation of promastigote form. The kinetic properties of the pure enzyme were determined and the  $K_m$  values for glucose ( $K_m=66 \mu\text{M}$ ) and ATP ( $K_m=303 \mu\text{M}$ ) were comparable to those from hexokinase of *Trypanosoma cruzi*. *L. mexicana* hexokinase was able to use fructose ( $K_m=142 \mu\text{M}$ ), which reflects the condition found in the insect host. In contrast with hexokinases from other trypanosomatids, the enzyme exhibited a moderate sensitivity to inhibition by glucose 6-phosphate. This inhibition was competitive with respect to both ATP and glucose, indicating that an allosteric site for glucose 6-phosphate does not exist in this enzyme. The enzyme was also inhibited by inorganic pyrophosphate, the inhibition being higher than that observed for *T. cruzi* enzyme. As expected, the enzyme was localized, by immunofluorescence analysis, in glycosomes and is present in both promastigotes and true amastigotes obtained from hamster lesion. Hexokinase specific activity increased with the aging of promastigote culture, and this increment was related to glucose consumption. However, the level of the hexokinase protein remains constant as determined by Western blotting. Several hypotheses are discussed to explain this result.

### Introduction

Parasites of the genus *Leishmania* belong to the family of trypanosomatidae and are the causative agents of leishmaniasis. This disease has a spectrum of clinical manifestations ranging from cutaneous lesion to visceral infection. In these parasites, the glycolytic metabolism is an important path for the generation of ATP in which the initial step is the irreversible ATP-dependent phosphorylation of glucose catalyzed by hexokinase (EC 2.7.1.1). This enzyme is sequestered, like other enzymes of glycolysis, in the glycosome, an organelle unique to these organisms (Hannaert et al. 2003). Hexokinase, in trypanosomatids, has been pointed out as drug target due to its difference with its homologous enzyme from the human host (Opperdoes and Michels 2001). In *Trypanosoma cruzi*, the causative agent of Chagas disease, this enzyme has been shown to be sensible to inorganic pyrophosphate (PPi; Cáceres et al. 2003), and as a consequence, biphosphonates act as potent inhibitors of this enzyme (Hudock et al. 2006). Moreover, biphosphonates have been signaled as possible therapy in Chagas disease since their action is very selective (Hudock et al. 2006).

Hexokinase has been well-characterized kinetically in *T. cruzi* and *Trypanosoma brucei*. However, in *Leishmania* many characteristics of this enzyme that allow designing a rational strategy of selective inhibition are unknown. A molecular characterization through the coding sequence in several species of *Leishmania* including *Leishmania mexicana* has been recently performed and, like *T. cruzi* and *T. brucei*, this enzyme from *Leishmania* species are 50-kDa type hexokinases (Umasankar et al. 2005). In this work, we have purified to homogeneity hexokinase from *L. mexicana* in order to study its kinetic properties. Additionally, as part of an effort to better understand the role of hexokinase on the control of glucose metabolism in this

M. A. Pabón · A. J. Cáceres · M. Gualdrón · W. Quiñones ·  
L. Avilán · J. L. Concepción (✉)  
Laboratorio de Enzimología de Parásitos,  
Centro de Ingeniería Genética, Facultad de Ciencias,  
Universidad de Los Andes, La Hechicera,  
Mérida 5101, Venezuela  
e-mail: concepci@ula.ve

parasite, we studied the expression of this enzyme throughout the growth curve in relation to the glucose consumption. Finally, we performed a subcellular distribution and fluorescence microscopy analyses to corroborate the localization of this enzyme in glycosome in both promastigote and amastigote forms.

## Materials and methods

### Growth of *L. mexicana*

Promastigotes of the *L. mexicana* strain AZV (Pérez et al. 1979) were cultivated at 28°C in Schneider medium supplemented with 20% fetal bovine serum. Hamster footpad lesions were used as sources of amastigotes for isolation and for maintenance of the parasites. An amastigotes-enriched preparation was obtained from the lesion as described elsewhere (Calcagno et al. 2002).

### Subcellular fractionating of promastigotes of *L. mexicana*

Parasites were harvested in exponential phase by centrifugation and washed twice in buffer A (20 mM Tris-HCl, pH 7.2; 5 mM EDTA; 10 mM KH<sub>2</sub>PO<sub>4</sub>; 20 mM KCl; and 225 mM sucrose) and once in buffer B (25 mM Tris-HCl, pH 7.6; 1 mM EDTA; and 250 mM sucrose containing a cocktail of protease inhibitors: 10 µM leupeptin, 50 µg/ml trypsin inhibitor, 1 mM benzamidine, 50 µM phenylmethylsulfonyl fluoride, 100 µM tosyl-L-lysine chloromethyl ketone, 0.2 µM pepstatin, 1 µM E-64, 1 µM chemostatin, 1 µM bestatin). Cells were then broken by abrasion with silicon carbide (mesh 200). The resulting suspension was centrifuged at 150×g for 3 min to eliminate the silicon carbide. Then the homogenate was submitted to differential centrifugation. First, the homogenate was centrifuged at 3,000×g for 10 min to remove unbroken cells and nuclei, and then the supernatant was centrifuged at 5,000×g for 10 min to obtain the large granular fraction. With the following centrifugation at 33,000×g for 20 min, a small granular fraction was obtained. The supernatant, after this step, was centrifuged at 105,000×g for 1.5 h to obtain the microsomal fraction. The supernatant after this last step corresponds to the cytosolic fraction. All centrifugation steps were performed at 4°C.

### Purification of hexokinase

The small granular fraction (glycosome-enriched fraction) obtained from the differential centrifugation was used to purify hexokinase. This fraction was solubilized in buffer C (10 mM Tris-HCl, pH 7.5; 0.1% Triton X-100; and 150 mM NaCl) and centrifuged at 33,000×g for 20 min.

The supernatant was loaded on a sepharose CL-6B column (2.5×75 cm) equilibrated with 25 mM Tris, pH 7.5; 1 mM MgCl<sub>2</sub>; and 150 mM NaCl. After elution, the fractions displaying hexokinase activity were pooled, dialyzed, and loaded on a column of DEAE-52 cellulose (Whatman) equilibrated with 25 mM Tris-HCl, pH 7.5, and 1 mM MgCl<sub>2</sub>. Hexokinase was eluted in the wash step. The enzyme was stored at 4°C in the presence of 5% glycerol and 1 mM dithiothreitol.

### Enzyme assays and kinetic analysis

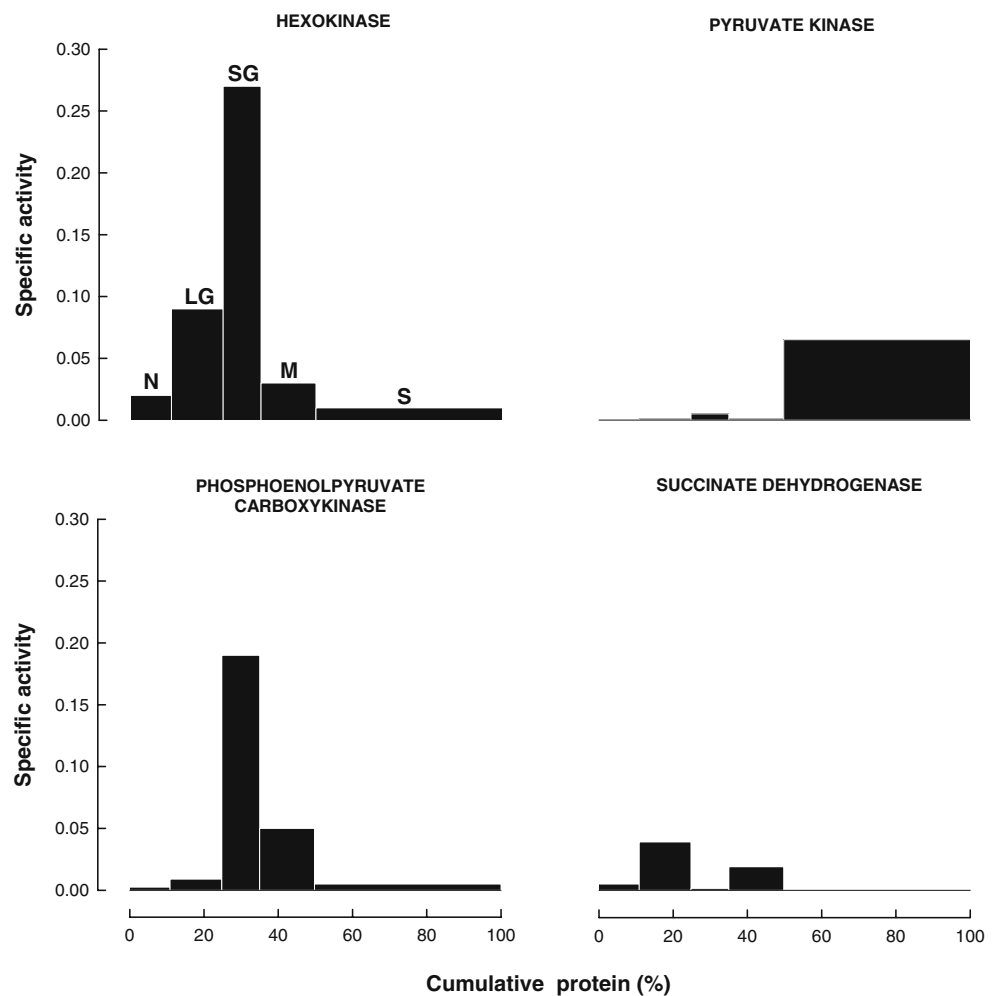
Hexokinase activity was measured spectrophotometrically using a coupled assay with glucose 6-phosphate dehydrogenase. The velocity of the reaction was followed by the apparition of NADPH at 340 nm. The assay contained 300 mM Tris-HCl, pH 8.5; 25 mM NaCl; 2 mM MgCl<sub>2</sub>; 2 mM ATP; 3 mM glucose; 0.8 mM NADP<sup>+</sup>; and 1 U glucose 6-phosphate dehydrogenase from *Leuconostoc mesenteroides* (Sigma, USA). When fructose was used as substrate, the activity was measured following the ADP production as described elsewhere (Cáceres et al. 2003). The reaction was started with enzyme. For the measurement of kinetic parameters, the substrate concentrations were varied from 0.017 to 0.2 mM for glucose and fructose and 0.05 to 1 mM for ATP. Inhibition studies were performed varying the concentration of fructose (0–0.6 mM), ADP (0–1 mM), glucose 6-phosphate (0–1.5 mM), and inorganic pyrophosphate (PPI; 0–0.3 mM). The effect of the pH was studied using Tris-HCl buffer for the pH range from 7.5 to 10 and TES buffer for the pH range from 6 to 8.5. The divalent cation requirement was assayed by varying the concentration of MgCl<sub>2</sub>, MnCl<sub>2</sub>, CuCl<sub>2</sub>, ZnCl<sub>2</sub>, CoCl<sub>2</sub>, or NiCl<sub>2</sub>. One unit of hexokinase activity is defined as the amount of the enzyme that catalyzes the production of 1 µmol of glucose-6-phosphate or ADP per minute. When cell suspension was used to measure hexokinase, parasites were resuspended in buffer C.

The activity of other enzymes was assayed as described previously: pyruvate kinase, EC 2.7.1.40 (Adroher et al. 1990); phosphoenolpyruvate carboxykinase, EC 4.1.1.4.9 (Urbina 1987); and succinate dehydrogenase, EC 1.3.9.1 (Köhler and Bachmann 1980).

### Glucose, ammonium, and protein determinations

Glucose and ammonium concentration in the growth medium was measured by an enzymatic method (Bergmeyer et al. 1974) using hexokinase and glutamate dehydrogenase (NAD<sup>+</sup>), respectively. Proteins were quantitatively assayed by the Lowry's method as described by Schacterle and Pollack (1973) using bovine serum albumin as the standard.

**Fig. 1** Subcellular distribution of hexokinase and marker enzymes obtained by differential centrifugation of a homogenate of *L. mexicana* promastigotes. The different fractions are shown in the order of their isolation from left to right. *N* Nuclear, *LG* large granular, *SG* small granular, *M* microsomal, *S* final supernatant corresponding to the cytosolic fraction. The *abscissa* indicates the cumulative protein content



#### Sodium dodecyl sulfate polyacrylamide gel electrophoresis and Western blotting

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970). For Western blotting experiments, proteins were transferred to polyvinylidene difluoride membranes. The membrane was blocked with phosphate-buffered saline (PBS) containing 5% casein and incubated with rabbit anti-hexokinase antibody raised against the *T. cruzi* enzyme for 1 h at room

temperature. After three washings with PBS, the membrane was incubated with peroxidase-conjugated secondary antibody and the labeled proteins were subsequently revealed by adding diaminobenzidine and  $H_2O_2$ .

#### Immunofluorescence

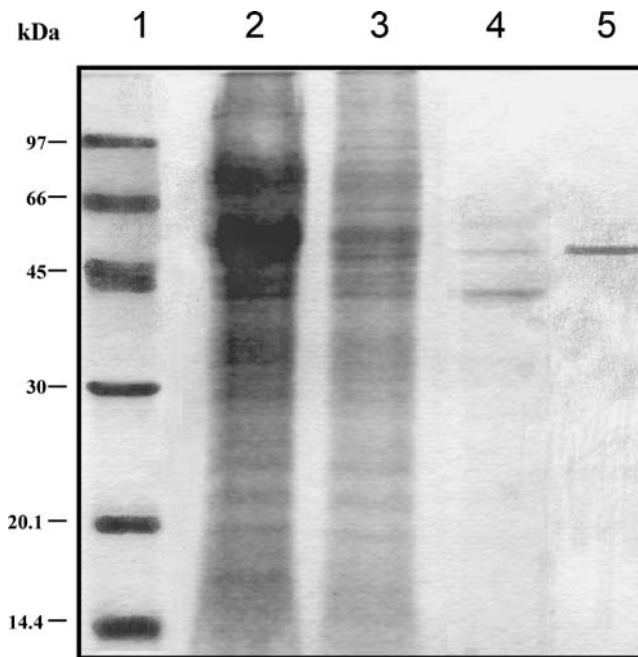
Promastigotes and the amastigote-enriched preparation were fixed with 4% (v/v) formaldehyde and allowed to adhere to poly-(L-lysine)-coated slides. Cells were then

**Table 1** Purification of *Leishmania mexicana* hexokinase

Fraction	Volume (ml)	Total activity <sup>a</sup> (U)	Total protein (mg)	Recovery (%)	Specific activity (U mg <sup>-1</sup> )	Fold purification
Homogenate	45.5	108.00	441.08	100	0.24	—
GEF <sup>b</sup>	6.0	47.00	52.71	44	0.89	3.71
Sepharose CL-6B	85.0	28.60	5.27	27	5.42	22.58
DEAE-52	88.0	27.26	1.06	25	25.71	107.12

<sup>a</sup> One unit is defined here as 1  $\mu$ mol NADP<sup>+</sup> reduced per minute at 25°C.

<sup>b</sup> Glycosome-enriched fraction.



**Fig. 2** Purification of *L. mexicana* hexokinase as followed by SDS-PAGE. Lane 1 Molecular mass marker, lane 2 small granular fraction (glycosome-enriched fraction), lane 3 small granular fraction solubilized with 0.1% Triton X-100 and 150 mM NaCl, lane 4 sepharose CL-6B step, lane 5 DEAE-cellulose flowthrough

permeabilized with 0.2% (v/v) Triton X-100 and washed with PBS. Cells were incubated with PBS containing 3% bovine serum albumin and 50 mM ammonium chloride for 30 min, and washed again with PBS. Then, the cells were incubated with anti-*T. cruzi* hexokinase, dilution 1:40 for 1 h, rinsed with PBS and incubated for 1 h with goat anti-rabbit IgG conjugated with Cy3. The samples were examined using an Olympus fluorescence microscope.

## Results

Hexokinase is expected to be localized mainly into the glycosome of all the trypanosomatids (Hannaert et al. 2003). For that reason, we performed a differential centrifugation to obtain a glycosome-enriched fraction as initial material to start the purification of this enzyme. After differential

centrifugation of the crude extract of the parasite, the hexokinase activity as well the activity of some marker enzymes was measured in each fraction. As shown in Fig. 1, the activity of hexokinase was found in the small granular fraction associated to the enzyme phosphoenolpyruvate carboxykinase that also has been previously shown to be located in the glycosome (Mottram and Coombs 1985). After solubilization with Triton X-100 and NaCl 150 mM, the small granular fraction was submitted to filtration chromatography (Sepharose CL-6B) and anion exchange chromatography (DEAE-cellulose), as indicated in the “Materials and methods,” to purify hexokinase. In the DEAE-cellulose step, the enzyme was recovered in the flowthrough of the column due to its alkaline isoelectric point (Umasankar et al. 2005). The results of the purification procedure are summarized in Table 1. The final preparation obtained was purified 107-fold as compared with the initial cell homogenate. The specific activity of the purified enzyme was 25.7 U/mg with ATP and glucose as substrates. The purified enzyme was homogenous by SDS-PAGE (Fig. 2) with a molecular mass of 53 kDa similar to nonvertebrate hexokinases and vertebrate hexokinase IV (Wilson 1995; Cárdenas et al. 1998; Cáceres et al. 2003).

Hexokinase from *L. mexicana* displayed an optimum pH around 8.5. The kinetic constants of pure hexokinase were determined for glucose, fructose, and ATP according to Lineweaver-Burk and are summarized in Table 2. *L. mexicana* hexokinase exhibited in all cases apparent Michaelis–Menten kinetics. The  $K_m$  for glucose and ATP were comparable to those reported for the enzyme from *T. cruzi* (Cáceres et al. 2003). However, contrary to the *T. cruzi* enzyme and similarly to other hexokinases (Cárdenas et al. 1998), *L. mexicana* hexokinase was able to use fructose. The  $K_m$  value for fructose (0.142 mM) indicates that this enzyme has a low specificity toward glucose. This  $K_m$  value is lower than those for fructose of nonspecific hexokinases from other sources (Colowick 1973). Magnesium was essential for the activity of *L. mexicana* hexokinase. The enzyme was unable to use any of the other divalent cations tested.

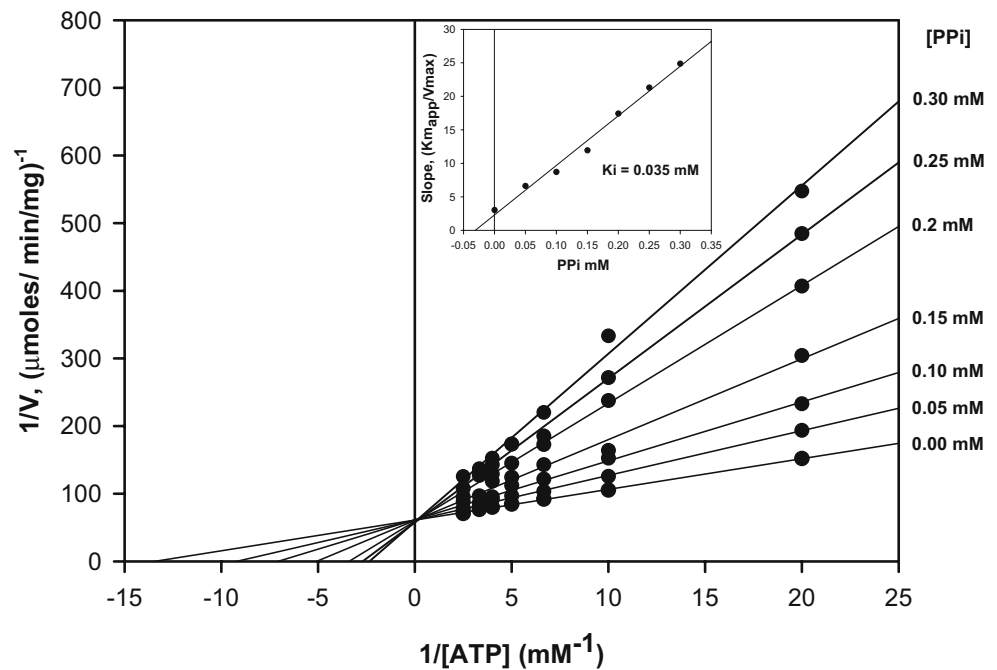
Hexokinases from other trypanosomatids like *T. brucei* (Willson et al. 2002) and *T. cruzi* (Cáceres et al. 2003) lack

**Table 2** Kinetic characteristics of *Leishmania mexicana* hexokinase

Activity pH optimum	Kinetic constant								
	Km (mM)			Kcat (min <sup>-1</sup> )	Kcat/Km (min mM <sup>-1</sup> )	K <sub>i</sub> (mM)			
	Glucose	Fructose	ATP			Fructose	G6-P	ADP	PPi
8–9	0.066	0.142	0.303	113968	1.72 × 10 <sup>6</sup>	(1) 0.230, (2) –	0.430 0.700	– 0.723	– 0.035

(1) denotes inhibition with respect to glucose and (2) denotes inhibition with respect to ATP.

**Fig. 3** Inhibition of *L. mexicana* hexokinase by inorganic pyrophosphate (PPi). Double reciprocal plot of velocity vs ATP concentration in the presence of the indicated concentrations of PPi. The  $K_i$  value was calculated from the slopes in each curve (*inset*)

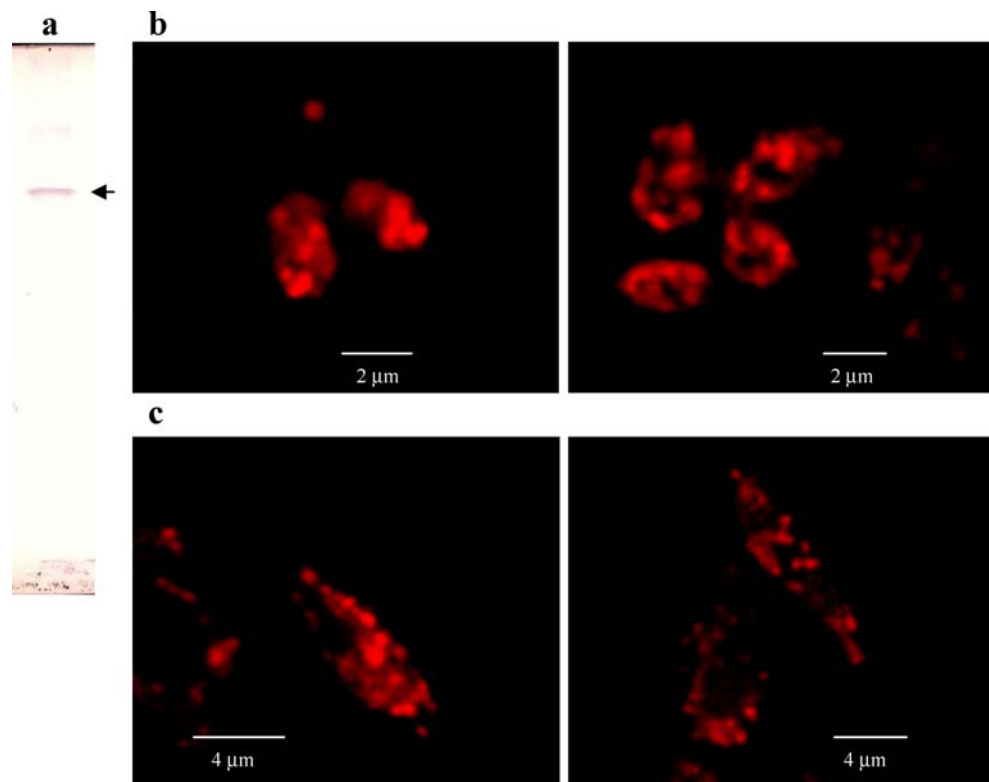


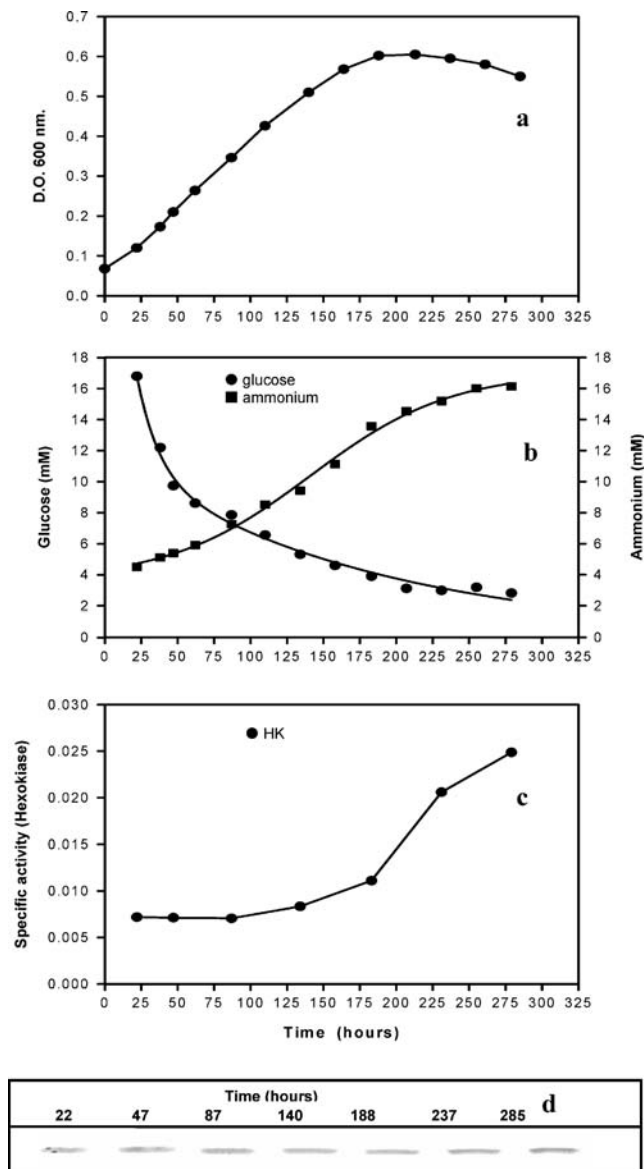
activity regulation by glucose 6-phosphate as the majority of 50-kDa type hexokinases. In the case of *L. mexicana* hexokinase, a moderate competitive inhibition by this product was obtained with a  $K_i$  of 0.43 and 0.7 mM for glucose and ATP, respectively (Table 2). The fact that this inhibition was competitive with respect to glucose indicates

that this inhibition is not the result of binding to an allosteric site of the enzyme.

Interestingly, *L. mexicana* hexokinase, similar to *T. cruzi* hexokinase, was inhibited by PPi with a  $K_i$  of 35  $\mu$ M with respect to ATP (Fig. 3). *L. mexicana* hexokinase was 14 times more sensitive to PPi than *T. cruzi* hexokinase

**Fig. 4** Immunofluorescence of *L. mexicana* promastigotes and amastigotes. **a** Western blot of promastigote extract showing the specificity of the anti-*T. cruzi* hexokinase antibody. *Arrow* shows the band recognized by the antibody. **b, c** Formaldehyde-fixed and Triton X-100 permeabilized cells stained with anti-*T. cruzi* hexokinase antibody. promastigotes from culture (**b**). True amastigotes derived from hamster lesion (**c**)





**Fig. 5** Hexokinase activity as a function of time during the growth of *L. mexicana* promastigote culture. **a** Growth curve of *L. mexicana* parasites. **b** Levels of glucose and ammonium in the medium during the growth curve. **c** Hexokinase specific activity during the growth curve. **d** Western blot of extract of parasite obtained in the indicated time. The antibody used was raised against *T. cruzi* hexokinase

( $K_i=500 \mu\text{M}$ ; Cáceres et al. 2003), and in the case of *L. mexicana*, the inhibition was competitive. Hexokinase did not present activity with PPI as substrate.

Hexokinase would be an interesting protein for designing a rational strategy of selective inhibition only if it is expressed in amastigote form. Umasankar et al. (2005) reported that hexokinase is poorly expressed in this form as determined by reverse transcription-polymerase chain reaction analysis. To know whether hexokinase is present in amastigote form, we performed an immunofluorescence experiment in amastigote and promastigote forms using

anti-*T. cruzi* hexokinase. This antibody recognized one band principally in a Western blot of promastigote extract corresponding to the molecular size of hexokinase (Fig. 4a). As shown in Fig 4b,c, glycosomes were clearly stained by anti-*T. cruzi* hexokinase in both promastigote and amastigote forms. The number of glycosome in amastigote form is fewer than in promastigotes, corroborating a previous report (Coombs et al. 1986). However, hexokinase is present indicating active glycolysis.

Hexokinase expression could be regulated in this parasite. It has been previously reported in a cutaneous leishmaniasis strain of *L. infantum* (Louassini et al. 1999) that the specific activity increases from the logarithmic phase to stationary phase. To study the hexokinase activity during promastigote growth in *L. mexicana*, parasite samples at different days were assayed for enzyme activity. Additionally, glucose and ammonium were measured in the medium. As Fig. 5a,c shows, hexokinase specific activity increases continuously during promastigote growth, reaching maximum values on late stationary phase, which is about threefold higher than the specific activity measured on day 1. In our culture conditions, *L. mexicana* promastigotes consumed both glucose and amino acids, with the latter measured by ammonium excretion (Fig. 5b). The increase in hexokinase activity is coincident with the consumption of glucose as the parasites progress to the stationary phase (Fig. 5b). However, Western blotting analysis using parasites obtained in different points of the curve did not show an increase in the level of hexokinase protein (Fig. 5d).

## Discussion

Hexokinase enzyme has been previously well-studied in *T. brucei* (Willson et al. 2002) and *T. cruzi* (Racagni and Machado De Doménech 1983; Cáceres et al. 2003). However, in *Leishmania* parasite, only limited characterizations have been performed principally related to its subcellular localization (Coombs et al. 1982; Hart and Opperdoes 1984; Mottram and Coombs 1985) and its specific activity in cellular extract (Cazzulo et al. 1985). In the present study, hexokinase was purified to homogeneity from promastigotes of *L. mexicana* and the kinetic properties of this enzyme were determined. Several interesting properties of this enzyme emerged from this study. (1) This enzyme has the ability to use fructose. In this respect, it differs from *T. cruzi* hexokinase, which is specific for glucose. This feature might be related to adaptation to different environments. *Leishmania* parasites, but not *T. cruzi*, have stages in their life cycle where fructose is available (*Leishmania* in the insect vector that can feed on nectar). (2) *L. mexicana* hexokinase exhibits a moderate sensitivity to inhibition (competitive vs ATP and glucose)

by the product glucose-6-phosphate. The inhibition by glucose-6-phosphate has been the subject of many studies in the 100-kDa type hexokinases and in a few 50-kDa type enzyme such as hexokinase from *Schistosoma mansoni* (Tielens et al. 1994; Armstrong et al. 1996), since this inhibitor binds to a discrete allosteric site, which has been defined by structural studies (Mulichak et al. 1998). In these cases, the inhibition is in a noncompetitive manner with respect to glucose. Thus, the result of competitive inhibition with respect to glucose in *L. mexicana* hexokinase confirms that this allosteric site does not exist in this enzyme. This result also confirms that all trypanosomatid hexokinases lack the glucose-6-phosphate regulatory site. The absence of allosteric regulation has been predicted by sequence analysis by Umasankar et al. (2005). The competitive inhibition by glucose-6-phosphate of *L. mexicana* hexokinase, in spite of being moderate, may have a role in the regulation of glycolysis in *Leishmania*. The implication of this regulation by glucose-6-phosphate needs further investigation. (3) *L. mexicana* hexokinase was inhibited by PPi, the inhibition being higher than that observed for *T. cruzi* hexokinase and, in the case of *L. mexicana* enzyme, the inhibition was clearly competitive. The physiological significance of this inhibition is not clear. However, PPi is formed inside the glycosome by several pathways such as  $\beta$ -oxidation of fatty acids, biosynthesis of purine and pyrimidine (Michels et al. 2000). Test of inhibition of this enzyme using analogues of PPi and biphosphonate can be performed, like in *T. cruzi* hexokinase, to develop therapeutic agents for leishmaniasis (Hudock et al. 2006).

As expected, hexokinase activity was exclusive to the glycosome-enriched fraction. Moreover, fluorescence microscopy analysis shows that glycosomes can be well-stained using anti-hexokinase antibody. This staining was well-observed in both promastigote and amastigote forms. This result clearly indicates that hexokinase is present in amastigotes. It has been suggested that the main source of energy in this stage is fatty acid metabolism (Coombs et al. 1982). However, glycolysis remains functional in amastigotes.

In agreement with the observations of Louassini et al. (1999), we also detected an increase in activity with the aging of culture and it was related to the glucose consumption. However, the immunoblotting analysis shows that this increase is not due to the synthesis of extra enzyme. Several hypotheses arise from this result. Hexokinase can change its specific activity due to a change in the metabolic condition product of glucose availability. This change might be related either to protein–protein association or posttranslational modifications. Other hypothesis to be tested is that measured hexokinase activity would be added to the activity of other enzymes such as glucokinase. It has been shown by the genome analysis of *Leishmania major* and *T. cruzi* that these parasites have a glucokinase (Opperdoes and Szikora

2006). This enzyme could be overexpressing with the aging of the culture.

In summary, the characteristics of hexokinase here reported allow the comparison of this enzyme with other hexokinases from trypanosomatids. It shares properties with the *T. cruzi* enzyme although some differences are notorious (i.e., inhibition by glucose-6-phosphate). The study of the structures of these enzymes will permit a better comprehension of these differences. The strong inhibition by PPi for *L. mexicana* hexokinase here reported will allow looking for strategies of therapy using PPi analogs such as biphosphonates.

**Acknowledgment** This study was supported by project C-1333-05-03-B CDCHT-ULA.

## References

- Adroher FJ, Osuna A, Lupianez JA (1990) Differential energetic metabolism during *Trypanosoma cruzi* differentiation. II. Hexokinase, phosphofructokinase and pyruvate kinase. *Mol Cell Biochem* 94:71–82
- Armstrong RL, Wilson JE, Shoemaker CB (1996) Purification and characterization of the hexokinase from *Schistosoma mansoni*, expressed in *Escherichia coli*. *Protein Expr Purif* 8:374–380
- Bergmeyer HU, Bernt E, Schmidt F, Stork H (1974) D-Glucose: determination with hexokinase and glucose-6-phosphate dehydrogenase. In: Bergmeyer HU (ed) *Methods of enzymatic analysis*, vol. 3. Academic, New York, pp 1196–1201
- Cáceres AJ, Portillo R, Acosta H, Rosales D, Quiñones W, Avilan L, Salazar L, Dubourdieu M, Michels PA, Concepción JL (2003) Molecular and biochemical characterization of hexokinase from *Trypanosoma cruzi*. *Mol Biochem Parasitol* 126:251–262
- Calcagno M, Avilan L, Colasante C, Berrueta L, Salmen S (2002) Interaction of different *Leishmania mexicana* morphotypes with plasminogen. *Parasitol Res* 88:972–978
- Cárdenas ML, Cornish-Bowden A, Ureta T (1998) Evolution and regulatory role of the hexokinases. *Biochim Biophys Acta* 1401:242–264
- Cazzulo JJ, Franke de Cazzulo BM, Engel JC, Cannata JJ (1985) End products and enzyme levels of aerobic glucose fermentation in trypanosomatids. *Mol Biochem Parasitol* 16:329–343
- Colowick S (1973) The hexokinases. In: Boyer PB (ed) *The enzymes*, vol. 9. Academic, New York, pp 1–48
- Coombs GH, Craft JA, Hart DT (1982) A comparative study of *Leishmania mexicana* amastigotes and promastigotes. Enzyme activities and subcellular locations. *Mol Biochem Parasitol* 5:199–211
- Coombs GH, Tetley L, Moss VA, Vickerman K (1986) Three dimensional structure of the *Leishmania* amastigote as revealed by computer-aided reconstruction from serial sections. *Parasitology* 92:13–23
- Hannaert V, Bringaud F, Opperdoes FR, Michels PA (2003) Evolution of energy metabolism and its compartmentation in Kinetoplastida. *Kinetoplastid Biol Dis* 2:11
- Hart DT, Opperdoes FR (1984) The occurrence of glycosomes (microbodies) in the promastigote stage of four major *Leishmania* species. *Mol Biochem Parasitol* 13:159–172
- Hudock MP, Sanz-Rodríguez CE, Song Y, Chan JM, Zhang Y, Odeh S, Kosztowski T, Leon-Rossell A, Concepción JL, Yardley V, Croft

- SL, Urbina JA, Oldfield E (2006) Inhibition of *Trypanosoma cruzi* hexokinase by bisphosphonates. *J Med Chem* 49:215–223
- Köhler P, Bachmann R (1980) Mechanisms of respiration and phosphorylation in *Ascaris* muscle mitochondria. *Mol Biochem Parasitol* 1:75–90
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685
- Louassini M, Foulquie MR, Benitez R, Adroher FJ (1999) Activity of key enzymes in glucose catabolism during the growth and metacyclogenesis of *Leishmania infantum*. *Parasitol Res* 85:300–306
- Michels P, Hannaert V, Bringaud F (2000) Metabolic aspects of glycosomes in trypanosomatidae—new data and views. *Mol Biochem Parasitol* 16:482–489
- Mottram JC, Coombs GH (1985) *Leishmania mexicana*: subcellular distribution of enzymes in amastigotes and promastigotes. *Exp Parasitol* 59:265–274
- Mulchak AM, Wilson JE, Padmanabhan K, Garavito RM (1998) The structure of mammalian hexokinase-1. *Nat Struct Biol* 5:555–560
- Opperdoes FR, Michels PA (2001) Enzymes of carbohydrate metabolism as potential drug targets. *Int J Parasitol* 31:482–490
- Opperdoes FR, Szikora JP (2006) In silico prediction of the glycosomal enzymes of *Leishmania major* and trypanosomes. *Mol Biochem Parasitol* 147:193–206
- Pérez H, Labrador F, Torrealba JW (1979) Variations in the response of five strains of mice to *Leishmania mexicana*. *Int J Parasitol* 9:27–32
- Racagni GE, Machado De Doménech EE (1983) Characterization of *Trypanosoma cruzi* hexokinase. *Mol Biochem Parasitol* 9:181–188
- Schacterle GR, Pollack RL (1973) A simplified method for the quantitative assay of small amounts of protein in biologic material. *Anal Biochem* 51:654–655
- Tielens AG, van den Heuvel JM, van Mazijk HJ, Wilson JE, Shoemaker CB (1994) The 50-kDa glucose 6-phosphate-sensitive hexokinase of *Schistosoma mansoni*. *J Biol Chem* 269:24736–24741
- Umasankar PK, Jayakumar PC, Shouche YS, Patole MS (2005) Molecular characterization of the hexokinase gene from *Leishmania major*. *J Parasitol* 91:1504–1509
- Urbina JA (1987) The phosphoenolpyruvate carboxykinase of *Trypanosoma (Schizotrypanum) cruzi* epimastigotes: molecular, kinetic, and regulatory properties. *Arch Biochem Biophys* 258:186–195
- Wilson JE (1995) Hexokinases. *Rev Physiol Biochem Pharmacol* 126:65–198
- Willson M, Sanejouand YH, Perie J, Hannaert V, Opperdoes F (2002) Sequencing, modeling, and selective inhibition of *Trypanosoma brucei* hexokinase. *Chem Biol* 9:839–847