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Glycosidases in *Leishmania* and Their Importance for *Leishmania* in Phlebotomine Sandflies with Special Reference to Purification and Characterization of a Sucrase

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GONTIJO, N. F., MELO, M. N., RIANI, E. B., ALMEIDA-SILVA, S., AND MARES-GUIA, M. L. 1996. Glycosidases in *Leishmania* and their importance for *Leishmania* in phlebotomine sandflies with special reference to purification and characterization of a sucrase. *Experimental Parasitology* **83,** 117–124. Culture forms of *Leishmania* (*Leishmania*) *amazonensis* (IFLA/BR/67/PH8) produce an extracellular enzyme that hydrolyzes sucrose molecules into their component monosaccharides. This is important because phlebotomine sand flies, the invertebrate hosts of *Leishmania,* ingest plant sap or aphid and coccid honeydew rich in sucrose between blood meals and *Leishmania* promastigotes cannot uptake sucrose. The sucrase was purified and characterized; its molecular weight, estimated by gel filtration chromatography and SDS–PAGE electrophoresis, was about 73 kDa. K_m and V_{max} measured with sucrose as substrate were respectively 4.4 m*M* and 6.9 μmole glucose.min^{-1} (mg sucrase)$^{-1}$, with maximum pH activity at pH 5.5. A series of natural and *p*-nitrophenyl-derived substrates were assayed, characterizing the enzyme as a highly specific β-D-fructofuranoside fructohydrolase. When 11 species of *Leishmania* and 7 genera of trypanosomatids were screened, only the species of the genus *Trypanosoma* did not produce an enzyme with saccharolytic activity. These data are in agreement with the fact that the latter vectors do not acquire sucrose or raffinose in their meals. Searching for glycolytic enzymes other than sucrase, we found an *N*-acetyl-β-D-galactosaminolytic activity. This *N*-acetyl-galactosaminidase, here described for the first time, might have a role in peritrophic membrane disruption. The importance of sucrase and *N*-acetyl-β-D-galactosaminidase in the *Leishmania* life cycle is discussed.

INDEX DESCRIPTORS AND ABBREVIATIONS: *Leishmania amazonensis;* trypanosomatids; glycosidases; sucrase purification; *N*-acetyl-β-D-galactosaminidase; *Leishmania* life cycle; phlebotomine sand flies; BSA, bovine serum albumin; FCS, fetal calf serum; PNP, *p*-nitrophenyl; FPLC, fast protein liquid chromatography; SDS–PAGE, sodium lauryl sulfate polyacrilamide gel electrophoreses; OVA, ovalbumin; Carb. A., carbonic anhydrase; E.B., elution buffer.

INTRODUCTION

Leishmania parasites are transmitted by blood-sucking phlebotomine sand flies. Sand flies become infected with *Leishmania* by feeding on infected vertebrate hosts. Once in the gut of the sand fly, *Leishmania* multiply and develop through various morphological stages before reaching the infective metacyclic form (reviewed by Sacks 1989 and Killick-Kendrick 1990).

The development of *Leishmania* promastigotes in the insect gut and their later transmission require adequate nutritional support (Molyneux *et al.* 1991; Schlein 1993). Initially it can be provided by the blood ingested during the infective bite and later by regular sugar meals. In fact, wild-caught phlebotomine sandflies commonly have sugars in their gut (Molyneux *et al.* 1991), and under experimental conditions, the percentage of potential transmitting females is significantly higher when they are maintained on a postbloodmeal diet of sucrose and albumin (Warburg and Schlein 1986).

Until recently there was little information about these sugar sources in nature. When the

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sand fly phytophagy hypothesis was tested on 19 different plants, *Phlebotomus papatasi* fed on sap from 8 of them. Feeding on aphid and coccid honeydew, rich in sugars, was also recorded (Schlein and Warburg 1986; Schlein 1986).

Considering the above data and the fact that *Leishmania* promastigotes utilize sucrose in a defined medium (M. N. Melo, Ph.D. Thesis, 1982), we assumed the existence of an enzyme produced by *Leishmania* that is capable of splitting sucrose molecules into their component monosaccharides. Recently Blum and Opperdoes (1994) showed the presence of saccharolytic activity in *Leishmania donovani* cultures.

Confirming our hypothesis, *Leishmania amazonensis* was able to produce a soluble extracellular sucrase. The enzyme was purified to homogeneity and characterized.

Concomitantly, we searched for other glycolytic enzymes that would be released in the medium and found an *N*-acetyl-galactosaminolytic activity described herein for the first time.

The possible role of these enzymes and their importance to the life cycle of *Leishmania* are discussed.

Material and Methods

Cell Cultures

Maintenance culture. Promastigotes of *L. amazonensis* (IFLA/BR/67/PH8) were grown at 240°C with shaking in a chemically defined medium (Melo *et al.* 1985) supplemented with type V bovine serum albumin (BSA Sigma) (6 g liter^{-1}), 5% fetal calf serum (FCS), and sucrose (2 g liter^{-1}) as the only carbohydrate source.

Culture for sucrase purification. Mid-log phase maintenance culture promastigotes were washed twice in fresh medium containing 11 m*M* sucrose without BSA and FCS. The washed cells were brought to 4×10^7 (cells) ml^{-1} in 200 ml of the same medium and were cultivated for 4 days as already described. Purification was achieved after centrifugation and filtration in 0.8-μm nitrocellulose filters.

Screening for Saccharolytic Activity among Different Species of Trypanosomatids

The species were grown in blood agar medium and defined medium (Melo *et al.* 1985) containing sucrose (2 g liter^{-1}) and 5% FCS (liquid phase). The saccharolytic activity was assayed in the crude culture supernatant as described below.

$^{14}CO_2$ Evolution

The experiment was carried out according to Mukkada *et al.* (1985). One milliliter of the maintenance culture medium was seeded with 1×10^7 promastigote cells. [U-^{14}C]Sucrose was added to a final concentration of 11 m*M* with a specific radioactivity of 0.045 μCi μmole^{-1}. The evolved $^{14}CO_2$ was trapped in a strip of filter paper soaked with saturated $Ba(OH)_2$ solution and detected by scintillography.

2-Deoxi-D-Glucose and Sucrose Uptake Measurements

These procedures were based on the quick filtration method (Zilberstein and Dwyer 1984). Mid-log phase promastigotes from maintenance culture were washed three times in 0.1 *M* potassium phosphate buffer, pH 7.0, plus 5 m*M* $MgSO_4$, brought to 4×10^8 cells ml^{-1}, and maintained in an ice bath until use. To six tubes containing 100 μl of washed promastigotes, 100 μl of ^{14}C-labeled carbohydrate was added—final concentration: 0.1 m*M*, 5 μCi μmole^{-1}—and the tubes were incubated at 25°C for 0.5, 1, 2, 5, 10 and 20 min, respectively. After incubation, 3 ml of ice-cold buffer was added to each tube and the contents were filtered through a 0.8-μm filter. The radioactivity incorporated by the cells was measured by scintillography.

Glycolytic Activity Assays

1. Assays upon natural substrates. The glycolytic activities upon natural substrates were measured by the determination of the concentration of free glucose or fructose released. Glucose concentration was measured using a glucose kit (from Analisa, Belo Horizonte, Brazil) based on the glucose oxidase–peroxidase method described by Trinder (1969). Fructose was measured according to Chaplin (1986): each fructose molecule reduces a NADP molecule in a series of enzymatically catalyzed reactions. The NADPH produced is measured spectrophometricaly at 340 nm.

The assays were performed in two steps:

i. The natural substrates at 25 m*M* (final concentrations) were incubated with the enzyme, at 37°C, in a total volume of 0.5 ml in 0.1 *M* sodium citrate buffer, pH 5.5.

ii. For glucose determination, 200 μl of incubation mixture was taken and added to 1300 μl of kit color reagent. After 15 min at 37°C the absorbance was measured at 505 nm. For fructose determination, 100 μl of incubated mixture was taken and the assay was carried out as described (Chaplin 1986).

2. Assays upon synthetic substrates. The glycolytic activity upon synthetic PNP-derived substrates was performed as described by Verpoorte (1972): 100 μl of culture supernatant was incubated with a PNP substrate in 0.1 *M* sodium citrate buffer, final volume 0.5 ml. After incubation at 37°C for 18 hr, 1 ml of glycine buffer (pH 10.5) was added. The

p-nitrophenol released was determined spectrophotometrically at 400 nm.

In any assay with crude culture supernatant, the supernatant was previously filtered in a Sephadex G50 column equilibrated with water to remove small molecules such as glucose, fructose, and sucrose.

Distribution of the Saccharolytic Activity among Culture Supernatant, Cytosol, and Cell Membranes

One milliliter of culture was microcentrifuged for 1 min. The supernatant was stored at 0°C and the cells were washed three times in 25 m*M* potassium phosphate buffer, pH 7.0, containing 1 m*M* $MgSO_4$ and 0.115 *M* NaCl. One milliliter of distilled water was added to the pellet and the hypoosmotically disrupted cells were submitted to two freezing–thawing cycles and microcentrifuged for 3 min. The supernatant, corresponding to the cytosol solution, was stored as already described. The membrane pellet was washed in distilled water and resuspended in 1 ml of water. Finally, 100-μl volumes of the supernatant, cytosol, and membrane suspensions were assayed for saccharolytic activity using the glucose kit. The incubation time was 2 hr. A blank was based on heat-inactivated material. The data were plotted as a percentage of the total saccharolytic activity in 100 μl of culture.

Sucrase Purification

The culture supernatant was rapidly passed through a Sephadex G50 column equilibrated with distilled water for desalting and was immediately lyophilized. The lyophilized material was chromatographed in a Sephadex G75 column (110 × 1.4 cm, 1.5 ml min^{-1}) equilibrated with 0.05 *M* citrate buffer, pH 5.5, 0.15 *M* NaCl, 0.02% azide. The fractions, corresponding to the second peak, were passed quickly through a Sephadex G50 column previously equilibrated with starting buffer: 0.01 *M* sodium phosphate, 0.01 M β-glycerolphosphate, 1% glycerol, 0.02% azide, pH 7.5. The equilibrated material was immediately chromatographed on Q Sepharose in a FPLC system (column 3 × 1 cm, 0.5 ml min^{-1}). The eluting buffer was the same as already described, plus 0.25 *M* NaCl.

Determination of Protein Concentration

The measurements were carried out by the Bradford method (Sedmak and Grossberg 1977) except when very small amounts of protein were available. In this case the concentration was estimated by the enzyme peak area relative to the total chromatographic area corresponding to the total protein applied in the column.

RESULTS

L. amazonensis promastigotes were grown in a medium containing [U-^{14}C] sucrose, which was metabolized, releasing $^{14}CO_2$. While [U-^{14}C]2-deoxyglucose was taken up with time, reaching a steady state level after 10 min (80 pmole by 10^6 cells), sucrose molecules are not directly taken up. Sucrose uptake, therefore, requires previous hydrolysis; the monossaccharides that were produced—glucose and fructose—apparently may be transported by the same carrier (Pastakia and Dwyer 1987) to be metabolized, producing heat (Mares-Guia *et al.* 1990).

About 94% of the sucrase produced by *Leishmania* is secreted into the culture medium. In fact, only a residual saccharolytic activity was found in the cytoplasm (4.5%) and in the membrane fraction (1.5%).

In searching for different glycolytic activities in the culture supernatant, a series of natural carbohydrates and synthetic PNP-carbohydrate derivatives were assayed as substrates. Among the natural substrates, only sucrose and raffinose were significantly hydrolyzed, although there was very weak activity upon maltose, threalose, and melibiose (Table I). The other natural substrates tested—palatinose, lactulose, turanose, lactose, and cellobiose—were not hydrolyzed. Other glycolytic activities were detected only upon PNP-*N*-acetyl-β-D-galactosaminide and PNP-*N*-acetyl-β-D-glucosaminide when using PNP derivatives. The *N*-acetyl-glucosaminolytic activity was de-

TABLE I
Glycolytic Activity of the Crude Culture Supernatant upon Some Carbohydrates

Substrate	Substrate structure	Relative activity (%)
Sucrose	Glc-α(1-2)β-Fru	100
Raffinose	Gal-α(1-6)-Glcα(1-2)β-Fru	53
Maltose	Glc-α(1-4)-Glc	3
Threalose	Glc-α(1-1)α-Glc	<1
Melibiose	Gal-α(1-6)-Glc	<1

Note. In this experiment, glycolytic activity is the ability to release or fructose moieties from any of the substrates listed above. Glc: glucose; Fru: fructose; Gal: galactose. A relative activity of 100% corresponds to the actual activity of 1 μg glucose (or fructose) released min^{-1} (4.5 μg protein)$^{-1}$.

scribed previously and its role was discussed (Schlein *et al.* 1992). At pH 5.0, the *N*-acetyl-glucosaminolytic activity was about 10 times higher than that of *N*-acetyl-galactosaminolytic. At pH 6.5, there was no *N*-acetyl-galactosaminolytic activity and the *N*-acetyl-glucosaminolytic activity was reduced to 30%. The other PNP derivatives tested were PNP-β-D-glucopyranoside, PNP-α-D-glucopyranoside, PNP-β-D-galactopyranoside, PNP-α-D-galactopyranoside, PNP-β-D-manopyranoside, PNP-β-D-xilopyranoside, PNP-β-D-fucopyranoside, PNP-α-D-fucopyranoside, and PNP-α-L-fucoside.

The sucrase was purified in two steps: gel filtration chromatography in Sephadex G75 (Fig. 1a) followed by an ion exchange chromatography in Q Sepharose (Fig. 1b) in which the purified enzyme was eluted when the overall buffer composition was 6% elution buffer plus 94% starting buffer. Table II describes the sucrase purification scheme. The sucrase molecular weight as estimated by gel filtration chromatography (Fig. 1a) and by SDS–PAGE electrophoresis (Fig. 2) was 73 and 70 kDa, respectively.

With sucrose as substrate, K_m and V_{max} were 4.4 m*M* (Fig. 3) and 6.9 μmole glucose $\cdot$ min^{-1} $\cdot$ (mg sucrase)$^{-1}$, respectively. The optimum pH was pH 5.5 (inset, Fig. 3).

In the first purification step the saccharolytic activity was divided into two distinct peaks (Fig. 1a). The peaks might represent isoenzymes or different grades of glycosylation of the same protein backbone. Dimerization was discounted since no saccharolytic activity was observed where the sucrase dimers should have eluted on a gel filtration chromatography in Sephadex G150. The K_m of the purified sucrase and the apparent K_m obtained with crude culture supernatant were about the same (data not shown).

The action of the purified sucrase upon sucrose, raffinose, maltose, and *p*-nitrophenyl-α-D-glucopyranoside indicated that it is a β-D-fructofuranoside fructohydrolase, corresponding to the group E.C. 3.2.1.26 (Table III).

Except for species of the genus *Trypano-*

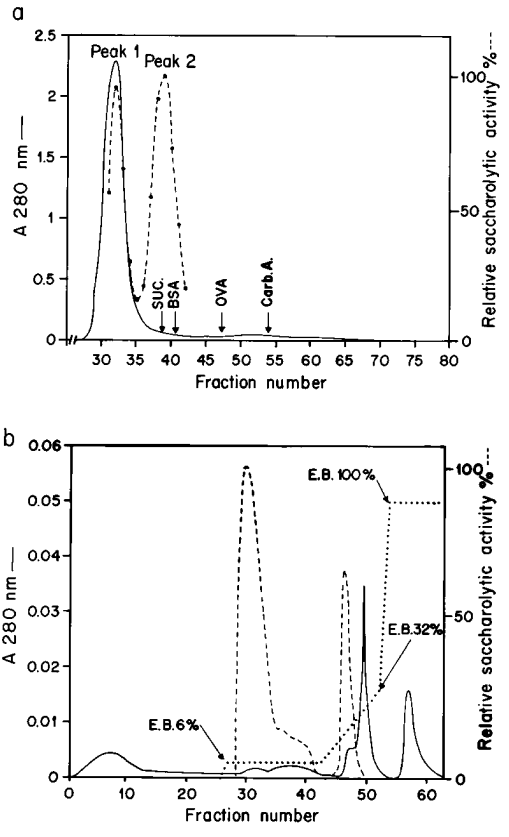

FIG. 1. (a) First purification step. The supernatant culture desalted by passing through Sephadex G50 and concentrated by lyophilization was chromatographed in a Sephadex G75 column, equilibrated with 0.05 *M* sodium citrate buffer, pH 5.5, containing 0.15 *M* NaCl and 0.02% azide. Saccharolytic activity was assayed as described under Materials and Methods. The column was previously standardized with BSA (66.7 kDa), Ova (ovalbumin, 45 kDa) and Carb. A. (carbonic anhydrase, 29 kDa). (· · ·) sucrase activity (suc., 73 kDa). (b) Second purification step. Ion exchange chromatography in Q Sepharose (FPLC System). The fractions corresponding to the second peak of saccharolytic activity were pooled and equilibrated in the starting buffer (0.01 *M* phosphate and 0.01 *M* β-glycerolphosphate buffer containing 1% glycerol and 0.02% azide, pH 7.5). The sucrase activity was eluted with the same eluting buffer (E.B.) as that mentioned above, with 0.25 *M* NaCl added. (—) Total protein (A280 nm); (---) relative saccharolityc activity%; (· · ·) percentage of the E.B. in mixture with starting buffer. In a and b, 100% relative activity corresponds to the actual activity of 8.7 μg glucose $\cdot$ min^{-1} $\cdot$ ml^{-1} and 0.7 μg glucose min^{-1} $\cdot$ ml^{-1}, respectively, in the most active peak fractions.

TABLE II
Sucrose Purification Scheme

	Total protein	Enzyme units	Specific activity	Enzyme yield	Purification factor
Culture crude supernatant	990 μg	497 mU	500 mU mg^{-1}	100%	1
After first step	34 μg	125 mU	3,670 mU mg^{-1}	25%	7.3
After second step	0.8 μg	20.2 mU	25,000 mU mg^{-1}	4%	50

Note. The enzyme was incubated with 25 m*M* sucrose for 1 hr at 37°C in citrate buffer 0.1 *M*, pH 5.5. 1 U, (1 μmole glucose).min^{-1}.(mg^{-1} sucrase) at 37°C.

soma, all others tested herein were able to split sucrose, as shown in Table IV.

Discussion

As described by Gemetchu (1974) an acellular peritrophic membrane is quickly secreted by sandflies' midgut cells around the ingested blood. Simultaneously, digestive enzymes are secreted to start the digestive process (Mahmood and Borovsky 1993). After an infective bloodmeal, the ingested parasites use the nutrients present in the digesting blood to support their initial transformation and multiplication. A few days after infection, the peritrophic membrane breaks down and the parasites migrate to the foregut where their development is completed (Killick-Kendrick 1990).

In the foregut, the parasite cells probably have as unique nutrients carbohydrates and other substances found in plant sap or aphid and coccid honeydew ingested after the infective bloodmeals. In fact, a regular sugar meal can enhance the number of promastigote cells developing in sand fly's gut (Sherlock and Sherlock 1961) and also the number of parasites egested by infected flies while bloodfeeding (Warburg and Schlein 1986).

The plant sap is very rich in sucrose, which may reach concentrations as high as 10 to 30% in 80% of the investigated plants (460 species in 552). Raffinose, the second most important sugar present in sap, reaches concentrations in the range of 2 to 30% in about 33% of these plants. On the other hand, free hexoses are

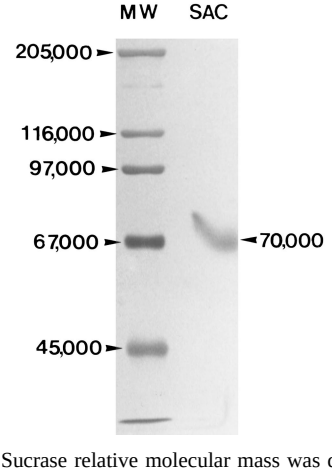

FIG. 2. Sucrase relative molecular mass was determined by electrophoresis in 7% SDS–PAGE. MW: relative molecular mass, SAC: *Leishmania* sucrase.

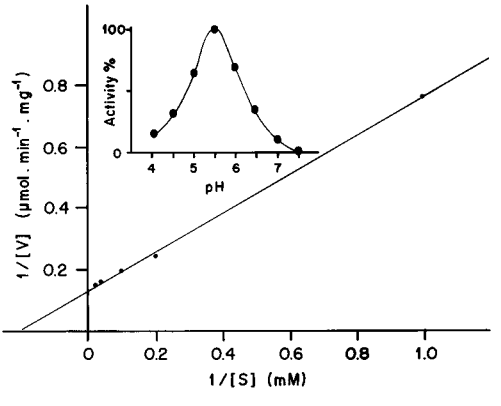

FIG. 3. Sucrase K_m and optimum pH. Using sucrose as substrate, K_m was estimated by a weighted linear regression; incubations were performed in 0.1 *M* sodium citrate buffer, pH 5.5, at 37°C for 3 hr. The enzyme was assayed in 0.1 *M* sodium citrate buffer, pH 4.0–6.5, and 0.1 *M* sodium phosphate buffer, pH 7.0–7.5, at 37°C for the optimum pH determination. The incubation lasted 2 hr.

TABLE III
Activity of the Purified Sucrase upon Natural and Synthetic Substrates

Substrate	Substrate structure	Relative activity (%)
Sucrose	Glc-α(1-2)β-Fru	100
Raffinose	Gal-α(1-6)-Glcα(1-2)β-Fru	30
Maltose	Glc-α(1-4)-Glc	4
p-N-phenyl-α-D-glucopyranoside	*p-N*-phenyl-α-D-Glc	0

Note. The capacity to release glucose or fructose moieties from any of the substrates was searched. Glc: glucose; Fru: fructose; Gal: galactose. A relative activity of 100% corresponds to the actual activity of 0.16 μg glucose (or fructose) released.min^{-1}.(0.37 μg sucrase)$^{-1}$. The deviations from the averages were around 7%.

found in very low concentrations and nitrogenous substances are commonly less concentrated than carbohydrates (Ziegler 1975).

The honeydew produced by aphids and coccids contains sucrose, glucose, fructose, and other carbohydrates regarded mainly as products synthesized from simple sugars through insect gut glycosidases (Auclair 1963).

Since promastigotes cannot take up sucrose and probably not raffinose either, the production of an extracellular sucrase is important for their development, even though phlebotomine sand flies produce at least one enzyme capable of splitting sucrose (Samie *et al.* 1990; Añez *et al.* 1989). In the early phase of the infection, when the parasites are arrested into the endoperitrophic space, small molecules such as sucrose and raffinose may easily pass through the peritrophic membrane where *Leishmania* sucrase can split them, thereby improving the chance of carbohydrate uptake by *Leishmania* cells.

The phlebotomine sucrase apparently is restricted to the midgut microvillar cell membranes (unpublished results) and the glucose and fructose produced by this enzyme, next to the midgut cells, would be promptly absorbed by the insect carriers, leaving little to be taken up by parasite cells within the endoperitrophic space.

In the foregut region, the promastigotes probably depend only on their own sucrase to split

TABLE IV
Saccharolytic Activity among Trypanosomatids

Species	Strain code	Saccharolytic activity
Leishmania (*Leishmania*) *amazonensis*	IFLA/BR/67/PH8	+
Leishmania (*L.*) *mexicana*	MHOM/BR/60/BH6	+
Leishmania (*L.*) *chagasi*	MHOM/BR/74/PP75	+
Leishmania (*L.*) *aethiopica*	MHOM/ET/72/L100	+
Leishmania (*L.*) *donovani*	MHOM/80/DD8	+
Leishmania (*L.*) *tropica*	MHOM/SU/74/K27	+
Leishmania (*L.*) *major*	MHOM/SU/73/5ASKH	+
Leishmania (*L.*) *infantum*	MHOM/TN/80/IPT1	+
Leishmania (*Viannia*) *guyanensis*	MHOM/BR/75/M4147	+
Leishmania (*V.*) *braziliensis*	MHOM/BR/84/LTB300	+
Leishmania (*V.*) *panamensis*	MHOM/PA/71/LS94	+
Crithidia fasciculata	ATCC 12 857	+
Phytomonas sp.	—	+
Herpetomonas samuelpessoai	ATCC 30 252	+
Leptomonas seymouri	ATCC 30 220	+
Sauroleishmania tarentolae	LV 414	+
Endotrypanum shaudinni	MCHO/BR/80/M6159	+
Trypanosoma cruzi	BERENICE 78-Y/4	−
Trypanosoma rangeli	P 19	−
Trypanosoma conorhini	—	−
Trypanosoma cruzi like	N 292/Clone	−

Note. The culture supernatants capable of releasing glucose from sucrose were considered positive.

sucrose. Thus, the *Leishmania* sucrase production could be the major enzyme involved in carbohydrate supply to the parasite.

Sucrose concentration in the sap (0.5 to 1.6 *M*) is more than sufficient to saturate *Leishmania* sucrase, whose K_m is 4.4 m*M*.

When activities other than the saccharolytic were assayed for natural (Table I) and synthetic PNP-derived substrates, we found two glycosidases: an *N*-acetyl-β-D-glucosaminidase, described earlier together with a chitinase (Schlein *et al*. 1992), and an *N*-acetyl-β-D-galactosaminidase described herein. Although information about the composition of phlebotomine peritrophic membrane is insufficient, it is possible that the *N*-acetyl-β-D-galactosaminidase described here may have a role in peritrophic membrane disruption. In fact, some hematophagous insects have *N*-acetyl-galactosamine in the composition of their peritrophic membranes (Billingsley 1990; Shahabuddin and Kaslow 1994) and the mosquito *Anopheles stephensi* has *N*-acetyl-galactosamine as its principal peritrophic membrane component (Berner *et al.* 1983).

The optimal pH for the action of the purified sucrase (inset of Fig. 3) is acid. Its acidic requirement is in agreement with the phlebotomine gut pH measured with indicator dyes (unpublished results) and with other enzymes excreted by *Leishmania* promastigotes, such as a chitinase, an *N*-acetyl-β-D-glucosaminidase (Schlein *et al.* 1992) and an acid phosphatase (Igl *et al* 1991). In addition, an acidic environment, *in vitro,* may promote promastigote transformation into metacyclic forms (Bates and Tetley 1993). These findings can promote a reformulation of the thoughts about the role of phlebotomine gut pH for *Leishmania* metacyclogenesis *in vivo.*

On the other hand, the sucrase activity obtained from *L. donovani* crude cultures was the same whether the assay was performed at pH 5.5 or pH 7.6 (Blum and Opperdoes 1994), but *L. amazonensis* sucrase had no activity at pH 7.5 (inset of Fig. 3).

Sucrose is hydrolyzed by two kinds of enzymes—β-fructofuranosidases (true sucrases) and certain α-glucosidases. The β-fructofuranosidases act on the fructose moiety, whereas the α-glucosidases attack the α-glucoside moiety. The characterization of an enzyme as a β-fructosidase is based on its ability to hydrolyze raffinose in addition to sucrose molecules (Myrback 1960). As *Leishmania* sucrase splits sucrose and raffinose and does not act on maltose or PNP-α-D-glucopyranoside, it should be classified as a true sucrase, that is, a β-D-fructofuranoside fructohydrolase, which belongs to the group E.C. 3.2.1.26.

Leishmania sucrase is highly specific—among 10 natural substrates tested, only sucrose and raffinose were hydrolyzed to any extent (Table I). It requires a substrate with a terminal β-D-fructofuranosyl residue and any change in the fructose carbon participating in the glycosidic linkage results in loss of activity. If the saccharolytic activity has a significant role in the *Leishmania* life cycle, as we strongly propose here, other related species should produce an enzyme like this. In fact all the *Leishmania* species and other related protozoans tested, except those of the genus *Trypanosoma,* produced sucrase (Table IV). These data are in agreement with the fact that the *Trypanosoma* vectors do not get sucrose or raffinose as meal.

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