Available online at www.sciencedirect.com
ScienceDirect
Acta Tropica 101 (2007) 54–60
ACTA TROPICA
www.elsevier.com/locate/actatropica

Trypanosoma cruzi strains, Tulahuen 2 and Y, besides the difference in resistance to oxidative stress, display differential glucose-6-phosphate and 6-phosphogluconate dehydrogenases activities

A.A. Mielniczki-Pereira [a], C.M. Chiavegatto [a], J.A. López [b], W. Colli [c], M.J.M. Alves [c], F.R. Gadelha [a,*]

[a] *Departamento de Bioquímica, IB, UNICAMP, Campinas, SP, Brazil*
[b] *Departamento de Parasitologia, ICB, USP, São Paulo, SP, Brazil*
[c] *Departamento de Bioquímica, Instituto de Química, USP, São Paulo, SP, Brazil*

Received 26 June 2006; received in revised form 24 November 2006; accepted 7 December 2006
Available online 16 December 2006

Abstract

The drugs currently available for Chagas'disease treatment are unsatisfactory due to limited efficacy and toxic side effects, making the search for more specific pharmacological agents a priority. The components of the *Trypanosoma cruzi* trypanothione-dependent antioxidant system have been pointed out as potential chemotherapeutic targets for the development of more specific drugs. To work properly, this system must have a current supply of NADPH, provided by glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD). Here, we compare two *T. cruzi* strains, Tulahuen 2 and Y, regarding growth rate, cytosolic tryparedoxin peroxidase (TcCPX) concentration and pentose phosphate pathway dehydrogenases activities. Tulahuen 2 cells show higher values as compared to the Y strain when the following parameters are compared: TcCPX concentration, resistance to H_2O_2, growth index and G6PD activity. Different patterns of G6PD and 6PGD activities were observed among strains along the growth curve and when cells were challenged with H_2O_2. These data reinforce the heterogeneity within *T. cruzi* populations and also the importance of G6PD in protecting the parasite against reactive oxygen species.

Keywords: *Trypanosoma cruzi*; Glucose-6-phosphate dehydrogenase; 6-Phosphogluconate dehydrogenase; Oxidative stress

1. Introduction

Living organisms are continuously subjected to reactive oxygen species (ROS) produced in oxidative metabolism, detoxification of xenobiotics or by the action of ultraviolet radiation. Organisms have developed different antioxidant defense systems to cope with ROS, which include antioxidant enzymes and low molecular weight antioxidants (Halliwell, 1999). *Trypanosoma cruzi* is the etiologic agent of Chagas'disease. The parasite trypanothione system, as compared to mammalian antioxidant mechanisms, has unique and peculiar features enabling to assign several proteins as potential selective drug targets (Flohé et al., 1999). Antioxidant enzymes work sequentially in dif-

* Corresponding author at: Universidade Estadual de Campinas, Depto. Bioquímica, IB, C.P. 6109, CEP 13083-970, Campinas, SP, Brazil. Tel.: +55 19 37886137; fax: +55 19 37886129.
E-mail address: frgad@unicamp.br (F.R. Gadelha).

0001-706X/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2006.12.001

ferent sub-cellular compartments of *T. cruzi* to promote hydroperoxide detoxification. All pathways converge to trypanothione that is reduced to $T(SH)_2$ by trypanothione reductase using NADPH (Shames et al., 1986). The flux of reducing equivalents from $T(SH)_2$ can go either to tryparedoxin or glutathione, which in turn can transfer electrons to peroxidases. Distinct peroxidases have been identified in *T. cruzi*: two peroxiredoxins, located in the cytosol (cytosolic tryparedoxin peroxidase—TcCPX) and in the mitochondria (mitochondrial tryparedoxin peroxidase—TcMPX), respectively, which efficiently detoxify H_2O_2 and small-chain organic hydroperoxides (Wilkinson et al., 2000); two glutathione-dependent peroxidases that detoxify fatty acid and phospholipid hydroperoxides, but not H_2O_2 (Wilkinson et al., 2002a), and an ascorbate-dependent hemoperoxidase (Wilkinson et al., 2002b). In spite of their complexity, the antioxidant pathways of *T. cruzi* render parasites less resistant to oxidative stress when compared to the mammalian host (Krauth-Siegel and Coombs, 1999).

To work properly, the trypanothione-dependent system must have a current supply of NADPH, provided by glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6-PGD), enzymes of the pentose phosphate pathway (PPP) (Barrett, 1997). G6PD is essential to mammalian cell survival when damage is produced by ROS. Targeted disruption of the housekeeping gene encoding G6PD in these cells revealed that G6PD is dispensable for pentose synthesis, but essential for defense against oxidative stress (Pandolfi et al., 1995). The PPP is operative in living epimastigotes and its activity increases in the presence of methylene blue, which oxidizes NADPH, a situation that mimics oxidative stress. Substantial levels of the PPP enzymes were found in amastigotes and trypomastigotes (cell cultured-derived trypomastigotes and metacyclic forms) that could be related to a possible higher exposure to oxidative stress inside the mammalian host (Maugeri and Cazzulo, 2004).

T. cruzi displays a remarkably high degree of both structural and functional intraspecific heterogeneity (Dvorak et al., 1988; Brisse et al., 1998), which could modulate pathogenicity, survival and adaptability (Brisse et al., 1998; Engel et al., 1990). Variations were observed within the *T. cruzi* strains regarding oxidative metabolism (Engel et al., 1990), rate of glucose catabolism *via* PPP (Mancilla and Naquira, 1964) and isoenzyme patterns (Goldberg and Pereira, 1983). In addition, differences in the participation of alternative pathways of carbohydrate metabolism were suggested to have some relationship to pathogenicity since in a more pathogenic strain (Tulahuen), PPP operates in a higher extent than in a less pathogenic one (Peruvian) (Mancilla and Naquira, 1964).

Herein, two strains with different resistance to the H_2O_2-generated oxidative stress were studied (Y and Tulahuen 2 strains). It has been found that parasites with a higher resistance to oxidative stress have a higher TcCPX content and G6PD activity, two enzymes that directly or indirectly participate in the trypanothione-dependent antioxidant system. The results also reemphasize the heterogeneity among *T. cruzi* strains and the relationship between resistance to oxidative stress and G6PD activity.

2. Materials and methods

2.1. Cell cultures

T. cruzi epimastigotes (Y and Tulahuen 2 strains) were grown in LIT medium, containing 20 mg l^{-1} hemin and 10% fetal bovine serum, as described (Castellani et al., 1967). After 5 days (early stationary phase), the cells were harvested by centrifugation (1000 × *g* at 4 °C) and washed once with phosphate buffered saline (PBS), pH 7.2. The number of cells ml^{-1} was determined using a Neubauer chamber.

2.2. Western blot

Epimastigote polypeptides, collected in the early stationary growth phase, were resolved by SDS-PAGE (90 μg protein/lane, determined by the biuret assay) (Gornall et al., 1949) and electroblotted to nitrocellulose membrane using the Xcell^{TM} mini cell system (Novex^{TM}). The blots were blocked with 3% non-fat milk and incubated with polyclonal antibodies raised against TcCPX from *Crithidia fasciculata* (dil. 1:100, overnight at 4 °C) (Luo et al., 2006). After washing, the membrane was incubated with the secondary antibody conjugated to phosphatase (1:2500 anti-mouse IgG, Sigma) and the reaction was detected by BCIP (5-bromo-4-chloro-3-indolyl phosphate) and NBT (nitro blue tetrazolium).

2.3. Determination of hydrogen peroxide concentration

H_2O_2 solutions were prepared daily assuming an extinction coefficient of 81 M^{-1} cm^{-1} at 230 nm (Carnieri et al., 1993).

2.4. Determination of IC_{50}

Parasites in early stationary phase (5.2×10^6 cells/ml) were incubated in the presence of different concentrations of H_2O_2 in PBS. After 30 min, cells were collected by centrifugation, resuspended in culture medium and the cell density determined after 5 days of growth, as described above. The H_2O_2 concentration (μM) that inhibited parasite growth by 50% (IC_{50}) was established as described previously (Kelly et al., 1993).

2.5. Glucose-6-phosphate and 6-phosphogluconate dehydrogenases assays

On the days specified, cells were collected by centrifugation, resuspended in PBS in the presence of a protease inhibitor cocktail (Cocktail Set III, Calbiochem). The amount of the cocktail inhibitor employed was calculated based on its leupeptin content and considering that 80 μM leupeptin inhibits Cys-proteases from 4×10^8 cells. The combined activities of G6PD and 6PGD were measured by the addition of 1×10^6 cells/ml to the reaction mixture (50 mM Tris–HCl, pH 7.6, 50 mM KCl, 0.1% Triton X-100, 250 μM $NADP^+$, 2 mM $MgCl_2$, 1 mM 6-phosphogluconate and 1 mM glucose-6-phosphate) and the course of $NADP^+$ reduction followed at 340 nm (Cronin et al., 1989). G6PD activity was obtained by subtracting the activity of 6PGD measured from the combined activities of 6PGD and G6PD. The same protocol was employed for the determination of 6PGD and G6PD activities after incubation with H_2O_2.

2.6. Statistical analyses

Statistical analyses were performed using the one-way ANOVA combined with Tukey post-test ($p < 0.05$ were considered significant).

3. Results and discussion

Previous studies from our laboratory have pointed out the importance of cytosolic tryparedoxin peroxidase on *T. cruzi* protection against fluctuating levels of ROS generated during physiological processes (Finzi et al., 2004). In order to continue our studies regarding *T. cruzi* oxidative stress, two strains with different resistance to oxidative stress were compared. The hydrogen peroxide concentration found to inhibit 50% of growth (IC_{50}) was 98.5 ± 1.7 μM for the Y strain and 133.1 ± 2.1 μM for the Tulahuen 2 strain. No significant differences could be established among strains regarding the number of cells and protein concentration (data not shown).

To characterize these cells regarding their ability to detoxify peroxides, TcCPX levels in protein extracts from both strains were determined by Western blot. As shown in Fig. 1, the Tulahuen 2 strain has a higher protein level when compared to the Y strain. Despite these differences, the ability to detoxify low concentrations of exogenous H_2O_2 (20 μM) detected by the phenol red method, was similar for both strains in the presence or absence of glucose (data not shown).

Based on the differences in resistance to the oxidative stress generated by H_2O_2 and due to the fact that NADPH is one of the components of the trypanothione-dependent antioxidant system, the NADPH production

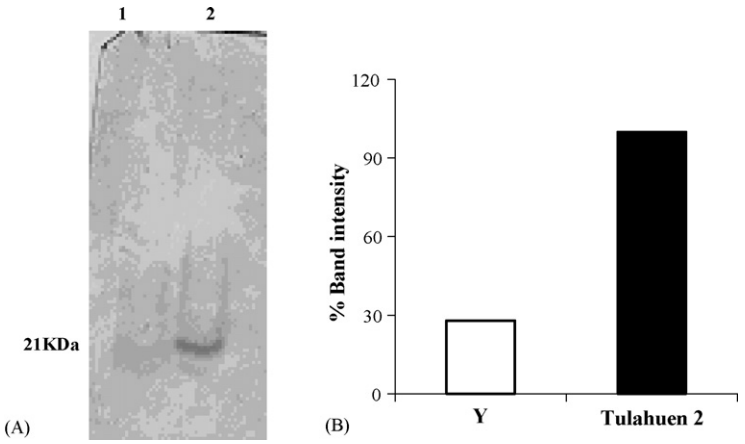

Fig. 1. Western blotting analysis of tryparedoxin peroxidase in *T. cruzi* cell extracts. Cell lysates and Western blotting were performed as described in Section 2. (A) Lane 1: Y cell extract and lane 2: Tulahuen 2 cell extract. (B) Percentage of band intensity as compared to the absorbance of lane 1. Data from Western blotting were analyzed by the ImageMaster® Total Lab program 1.0 version (Amersham Pharmacia Biotech). A representative of four independent experiments is shown.

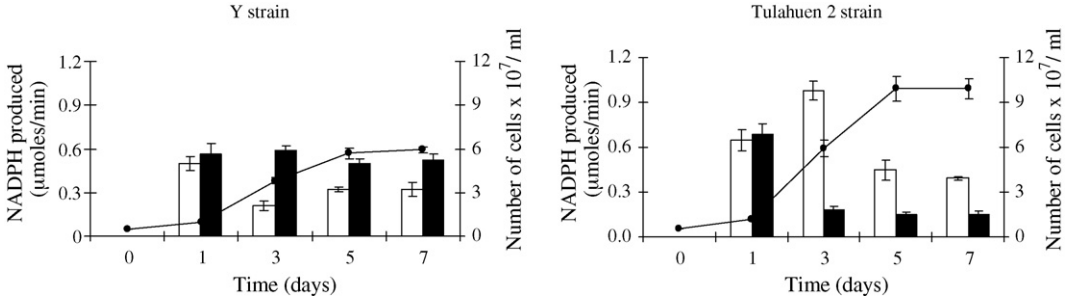

Fig. 2. G6PD and 6PGD activities along the growth curve of *T. cruzi*. Cells (5.2×10^6/ml) were incubated in culture medium and, on the days specified, the number of cells (●) was determined using a Neubauer chamber and PPP dehydrogenases activities, G6PD (empty bars) and 6PGD (black bars), were determined in 10^6 cells/ml as described in Section 2. Statistical analyses for enzyme activity—Y strain: G6PD on day 1 compared to all days, $p < 0.001$. 6PGD on day 1 compared to the others, $p > 0.05$ and third day compared to 5 and 7 days, $p < 0.001$ and $p < 0.01$, respectively); Tulahuen 2 strain: G6PD and 6PGD on day 1 compared to all days, $p < 0.001$. G6PD on fifth day compared to seventh day, $p > 0.05$. 6PGD on days 3, 5 and 7, $p > 0.05$. Under the experimental conditions employed, no NADPH production was detected in the absence of glucose-6-phosphate and 6-phosphogluconate. The means of six duplicate experiments performed on different days are shown.

by G6PD and 6GPD was measured along the growth curve of the parasite, corresponding to lag (day 1), log (day 3), early (day 5) and late stationary (day 7) phases (Fig. 2). Both strains reached the stationary phase after 5 days of incubation in the experimental conditions employed, although with a higher cell density in the case of Tulahuen 2 strain, since doubling time for Tulahuen 2 and Y strains were estimated as 29.99 ± 2.52 h and 38.40 ± 2.56 h, respectively (Toma et al., 2000). Growth indices were found to be 12.97 ± 1.06 and 7.97 ± 0.92 for Tulahuen 2 and Y strains, respectively (Martínez-Diaz et al., 2001).

G6PD activity varied along the growth curve in both strains (Fig. 2). In the Y strain, G6PD and 6PGD had similar activities in the lag phase. In the following phases a decrease in G6PD was observed: 58% (log phase) and 36% (early and late stationary phases), as compared to values in the lag phase. 6PGD activity remained almost the same along the growth curve (Table 1).

Tulahuen 2 strain showed a different pattern of PPP dehydrogenases activities. In the log phase, G6PD activity was 50% higher than the activity observed in the lag phase, reaching a value approximately five times higher than the other strain for the same period. Different from Y parasites, a drastic decrease in 6PGD activity (74%) was observed from log to stationary phase, but G6PD activity remained higher than 6PGD along the growth curve. In Tulahuen 2 strain, except for the lag phase, 6PGD activity was about 18-38% of G6PD activity. This lower 6PGD activity could lead to the accumulation of 6-glucono-γ-lactone-6-phosphate or 6-phosphogluconate (6PG). Accumulation of the first product is apparently not toxic to cells (Duffieux et al., 2000) and is less probable to occur due to its transformation to 6PG either spontaneously or by enzymatic reactions. Apparently, accumulation of 6PG is toxic for eukaryotic cells (Lobo and Maitra, 1982). Since Tulahuen 2 cells continue to grow despite the large difference between G6PD and 6PGD in the log phase, it is reasonable to suppose that the concentration of 6PG in that specific situation is not toxic for the cells.

These different patterns of enzyme activity observed among strains can be a result of variations between strains in kinetic parameters such as Km and Vmax, as

Table 1
NADPH production (μmol NADPH produced/min/10^6 cells) by G6PD and 6PGD during proliferation of *T. cruzi* epimastigotes

Day	Y strain			Tulahuen 2 strain		
	G6PD	6PGD	Total	G6PD	6PGD	Total
1	$0.50 \pm 0.05^*$	0.56 ± 0.07	1.06 ± 0.11	$0.64 \pm 0.07^*$	$0.69 \pm 0.07^*$	1.30 ± 0.12
3	0.21 ± 0.03	$0.59 \pm 0.03^*$	0.80 ± 0.05	$0.98 \pm 0.06^*$	0.18 ± 0.03	1.14 ± 0.12
5	0.32 ± 0.02	0.50 ± 0.03	0.81 ± 0.06	0.45 ± 0.07	0.15 ± 0.02	0.56 ± 0.06
7	0.32 ± 0.05	0.52 ± 0.04	0.84 ± 0.09	0.39 ± 0.01	0.15 ± 0.02	0.54 ± 0.04

Cells (5.2×10^6 per ml) were inoculated in culture medium and on the days specified G6PD and 6PGD activities were determined essentially as described in Section 2.5. Statistical analyses: significant differences were observed only in ($^*p < 0.05$): Y strain—G6PD activity compared to all activities obtained during the growth curve; 6PGD when compared to stationary phase; Tulahuen 2 strain: G6PD and 6PGD activities compared to other phases; G6PD on log phase compared to the others.

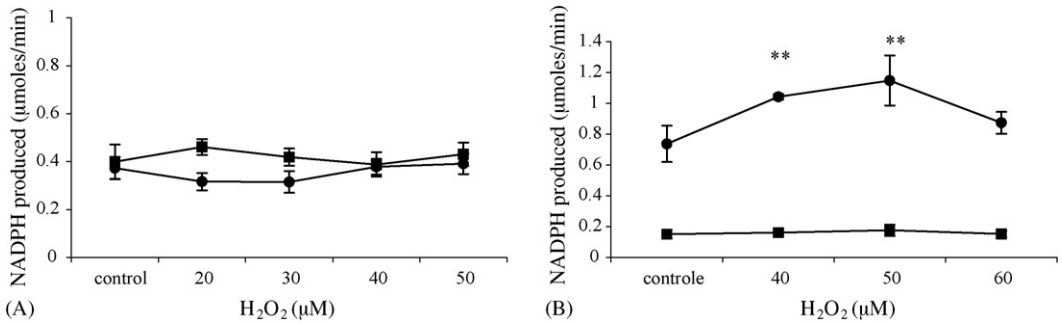

Fig. 3. G6PD and 6PGD activity in *T. cruzi* after incubation in the presence of different H_2O_2 concentrations—Cells (5.2×10^6/ml) were incubated for 30 min, with different concentrations of H_2O_2, in PBS. Cells were collected by centrifugation and resuspended in PBS containing the protease inhibitor cocktail. G6PD (●) and 6PGD (■) activities were determined in 10^6 cells/ml as described in Section 2. Y strain (A)—statistical analyses: no significant differences were observed. Tulahuen 2 strain (B)—G6PD control compared to 40 and 50 μM H_2O_2 treated groups ($^{**}p < 0.001$). 6PGD control compared to all treated groups ($p > 0.05$).

reported for *Leishmania brasiliensis* where the G6PD Km for $NADP^+$ and glucose-6-phosphate is twice that observed in *L. donovani* (Berens et al., 1980). Lupiañez et al. also demonstrated that in *T. cruzi* metacyclic forms, G6PD Km for glucose-6-phosphate is 4.6 higher than in the epimastigote forms (1.06 and 0.23 mM, respectively). The epimastigote stage, where cell division takes place in the invertebrate host, has a higher G6PD activity than metacyclic parasites, conceivably to provide cells with NADPH and nucleotides for biosynthetic purposes, an activity less pronounced in the metacyclic stage (Lupiañez et al., 1987).

Another interesting fact is that along the growth curve of the strain with higher proliferation rate and less susceptibility to H_2O_2, i.e., Tulahuen 2, G6PD activity was higher than in the Y strain, especially in the log phase. We have also observed that cells overexpressing TcCPX had both higher G6PD activity and growth rate when compared to wild type cells (Finzi et al., 2004). In other eukaryotic cells, G6PD is important for proliferation since it provides NADPH for redox regulation (Berens et al., 1980) and this could also be the case of *T. cruzi*. Moreover, yeast mutants for G6PD were sensitive to H_2O_2, implying that PPP plays a special role in resistance to oxidative stress (Juhnke et al., 1996). Supporting this idea, G6PD null cells (mouse embryonic stem cells) are viable but have a reduced growth rate especially as cell density increases and are more sensitive to oxidative stress than the wild type (Pandolfi et al., 1995).

Since an increase in PPP activity was observed when cells were in a situation that mimics oxidative stress (Maugeri and Cazzulo, 2004), cells were challenged with H_2O_2 and the activities of G6PD and 6PGD were measured (Fig. 3). The H_2O_2 concentrations tested were those at half of IC_{50} or lower. For the Y strain (Fig. 3A) no significant differences were observed in G6PD and 6PGD activities, for all H_2O_2 concentrations tested (20–50 μM). An opposite behavior was observed in Tulahuen 2 cells submitted to the same treatment. G6PD activity was stimulated and had higher values than 6PGD (Fig. 3B).

In human cells exposed to H_2O_2 both G6PD activity and mRNA are rapidly and transiently enhanced suggesting that G6PD could be part of an inducible mechanism to protect cells against oxidative damage (Ursini et al., 1997). To test if this could occur with *T. cruzi* cells, the H_2O_2 incubation time was extended. As observed in 30 min, no changes in both PPP dehydrogenases were observed in Y cells increasing the incubation period (Table 2). On the other hand, in Tulahuen 2 cells,

Table 2
G6PD and 6PGD activities in *T. cruzi* (Y strain) after different incubation times in the presence of H_2O_2

Time (min)	G6PD		6PGD	
	Control	H_2O_2 treatment	Control	H_2O_2 treatment
30	0.37 ± 0.05	0.39 ± 0.04	0.40 ± 0.07	0.43 ± 0.05
90	0.38 ± 0.05	0.42 ± 0.06	0.51 ± 0.10	0.35 ± 0.05
150	0.33 ± 0.07	0.33 ± 0.05	0.46 ± 0.06	0.49 ± 0.04

Cells (5.2×10^6/ml) were incubated in PBS for different times in the presence of 50 μM H_2O_2. Enzyme activities were determined in 10^6 cells/ml as described in Section 2. Statistical analyses: controls compared to all $p > 0.05$.

Table 3
G6PD activity in *T. cruzi* (Tulahuen 2 strain) after different incubation times in the presence of H_2O_2

Time (min)	G6PD		6PGD	
	Control	H_2O_2 treatment	Control	H_2O_2 treatment
30	0.64 ± 0.06	1.04 ± 0.1*	0.14 ± 0.03	0.17 ± 0.04
90	0.73 ± 0.125	1.04 ± 0.16**	0.18 ± 0.04	0.15 ± 0.02
150	0.77 ± 0.155	0.94 ± 0.11	0.18 ± 0.01	0.17 ± 0.03

Cells (5.2×10^6/ml) were incubated in PBS for different times in the absence or in the presence of 50 μM H_2O_2. G6PD and 6PGD activities were determined in 10^6 cells/ml as described in Section 2. Statistical analyses: G6PD: H_2O_2-treated groups for 30 and 90 min related to each control: $^{*}p < 0.001$ and $^{**}p < 0.01$, respectively. For 150 min, there were no statistical differences as compared to control ($p > 0.05$); 6PGD: no significant differences were observed.

no significant differences were observed in 6PGD, but G6PD activity in cells exposed to H_2O_2, was higher than control cells upon 30 and 90 min after addition of H_2O_2 (Table 3). After 150 min no significant differences among control and treated cells were observed (Table 3).

The results depicted herein reveal that G6PD can be modulated, as it occurs with TcCPX (Finzi et al., 2004) upon exposure to H_2O_2-generated oxidative stress. As shown, the cells with a higher resistance to oxidative stress have a higher G6PD activity and a higher TcCPx content, two enzymes that directly or indirectly participate in the trypanothione-dependent antioxidant system.

Acknowledgements

Authors are indebted to Luis Henrique Gonzaga Ribeiro for excellent technical assistance and the Brazilian funding agencies FAPESP, CNPq and Capes for financial support and scholarship.

References

Barrett, M.P., 1997. The pentose phosphate pathway and parasitic protozoa. Parasitol. Today 13, 11–16.

Berens, R.L., Deutsch-King, L.C., Marr, J., 1980. *Leishmania donovani* and *Leishmania braziliensis*: hexokinase, glucose-6-phosphate dehydrogenase, and pentose phosphate shunt activity. Exp. Parasitol. 49, 1–8.

Brisse, S., Barnabé, C., Bañuls, A.L., Sidibé, I., Noel, S., Tibayrenc, M., 1998. A phylogenetic analysis of the *T. cruzi* genome project CL Brener reference strain by multilocus enzyme electrophoresis and multiprimer random amplified polymorphic DNA fingerprinting. Mol. Biochem. Parasitol. 92, 253–263.

Carnieri, E.G.S., Moreno, S.N.J., Docampo, R., 1993. Trypanothione-dependent metabolism in *Trypanosoma cruzi* different stages. Mol. Biochem. Parasitol. 61, 79–86.

Castellani, O., Ribeiro, L.V., Fernandes, F., 1967. Differentiation of *Trypanosoma cruzi* in culture. J. Protozool. 14, 447–451.

Cronin, C.N., Derek, P.N., Voorheis, P.H., 1989. The enzymes of the classical pentose phosphate pathway display differential activities in procyclic bloodstream forms of *Trypanosoma brucei*. FEBS Lett. 244, 26–30.

Duffieux, F., Roy, J.V., Michels, P.A.M., Opperdoes, F.R., 2000. Molecular characterization of the first two enzymes of the pentose-phosphate pathway of *Trypanosoma brucei*. J. Biol. Chem. 275, 27559–27565.

Dvorak, J.A., Engel, J.C., Leapman, R.D., Swyt, C.R., Pella, P., 1988. *Trypanosoma cruzi*: elemental composition heterogeneity of cloned stocks. Mol. Biochem. Parasitol. 31, 19–26.

Engel, J.C., Doyle, P.S., Dvorak, J.A., 1990. Isolate-dependent differences in the oxidative metabolism of *Trypanosoma cruzi* epimastigotes. Mol. Biochem. Parasitol. 39, 69–76.

Finzi, J.K., Chiavegatto, C.W.M., Corat, K.F., Lopez, J.A., Cabrera, O.G., Mielniczki-Pereira, A.A., Colli, W., Alves, M.J.M., Gadelha, F.R., 2004. *Trypanosoma cruzi* response to the oxidative stress generated by hydrogen peroxide. Mol. Biochem. Parasitol. 133, 37–43.

Flohé, L., Hecht, H.J., Steinert, P., 1999. Glutathione and trypanothione in parasitic hydroperoxide metabolism. Free Rad. Biol. Med. 27, 966–984.

Goldberg, S.S., Pereira, A.A.S., 1983. Enzyme variation among clones of *Trypanosoma cruzi*. J. Parasitol. 69, 91–96.

Gornall, A.G., Bardawill, C.J., David, M.M., 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177, 751–766.

Halliwell, B., 1999. Antioxidant defense mechanisms: from the beginning to the end (of the beginning). Free Radic. Res. 31, 261–272.

Juhnke, H., Krems, B., Kötter, P., Entian, K., 1996. Mutants that show increased sensitivity to hydrogen peroxide reveal an important role for the pentose phosphate pathway in protection of yeast against oxidative stress. Mol. Gen. Genet. 252, 456–464.

Kelly, J.M., Taylor, M.C., Smith, K., Hunter, K.J., Fairlamb, A.H., 1993. Phenotype of recombinant *Leishmania donovani* and *Trypanosoma cruzi* which overexpress trypanothione reductase. Eur. J. Biochem. 218, 29–37.

Krauth-Siegel, R.L., Coombs, G.H., 1999. Enzymes of parasite thiol metabolism as drug targets. Parasitol. Today 15 (10), 404–409.

Lobo, Z., Maitra, P.K., 1982. Pentose phosphate pathway mutants of yeast. Mol. Gen. Genet. 185, 367–368.

Luo, S., Fang, J., Docampo, R., 2006. Molecular characterization of *Trypanosoma brucei* P-type H^+-ATPases. J. Biol. Chem. 281, 21963–21973.

Lupiañez, J.A., Adroher, F., Vargas, A.M., Osuna, A., 1987. Differential behavior of glucose 6-phosphate dehydrogenase in two morphological forms of *Trypanosoma cruzi*. Int. J. Biochem. 19, 1085–1089.

Mancilla, R., Naquira, C., 1964. Comparative metabolism of ^{14}C-glucose in two strains of *Trypanosoma cruzi*. J. Protozool. 11, 509–513.

Martínez-Diaz, R., Escario, J.A., Nogal-Ruiz, J., Gómez-Barrio, A., 2001. Biological characterization of *Trypanosoma cruzi* strains. Mem. Inst. Oswaldo Cruz 96, 53–59.

Maugeri, D.A., Cazzulo, J.J., 2004. The pentose phosphate pathway in *Trypanosoma cruzi*. FEMS Microb. Lett. 234, 117–123.

Pandolfi, P.P., Sonati, F., Rivi, R., Mason, P., Grpsveld, F., Luzzatto, L., 1995. Targeted disruption of the housekeeping gene encoding glucose 6-phosphate dehydrogenase (G6PD): G6PD is dispensable for pentose synthesis but essential for defense against oxidative stress. EMBO J. 14, 5209–5215.

Shames, S.L., Fairlamb, A.H., Cerami, A., Walsh, C.T., 1986. Purification and characterization of trypanothione reductase from *Crithidia fasciculata*, a newly discovered member of the family of disulfide-containing flavoprotein reductases. Biochemistry 25, 3519–3526.

Toma, H.K., Cerávolo, I., Guerra, H., Steindel, M., Romanha, A.J., 2000. *Trypanosoma cruzi*: parasitemia produced in mice does not seem to be related to *in vitro* parasite-cell interaction. Int. J. Parasitol. 30, 593–597.

Ursini, M.V., Parrella, A., Rosa, G., Salzano, S., Martini, G., 1997. Enhanced expression of glucose-6-phosphate dehydrogenase in human cells sustaining oxidative stress. Biochem. J. 323, 801–806.

Wilkinson, S.R., Temperton, N., Mondragon, A., Kelly, J., 2000. Distinct mitochondrial and cytosolic enzymes mediate trypanothione-dependent peroxide metabolism in *Trypanosoma cruzi*. J. Biol. Chem. 275, 8220–8225.

Wilkinson, S.R., Taylor, M.C., Touitha, S., Mauricio, I.L., Meyer, D., Kelly, J., 2002a. TcGPXII, a glutathione-dependent *Trypanosoma cruzi* peroxidase with substrate specificity restricted to fatty acid and phospholipid hydroperoxides, is localized to the endoplasmic reticulum. Biochem. J. 364, 787–974.

Wilkinson, S.R., Obado, S.O., Mauricio, I.L., Kelly, J.M., 2002b. *Trypanosoma cruzi* express a plant-like ascorbate-dependent hemoperoxidase localized to the endoplasmic reticulum. Proc. Natl. Acad. Sci. U.S.A. 99, 13453–13458.