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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

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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-

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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).