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1 Short communication

2 The glycosomal-membrane associated phosphoglycerate kinase 3 isoenzyme A plays a role in sustaining the glucose flux in 4 *Trypanosoma cruzi* epimastigotes

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ABSTRACT

In *Trypanosoma cruzi* three isoenzymes of phosphoglycerate kinase (PGK) are found which are simultaneously expressed: the cytosolic isoenzyme PGKB as well as two glycosomal enzymes, PGKA and PGKC. In this paper, we show that PGKA in *T. cruzi* epimastigotes is associated to the glycosomal membrane; it is responsible for about 23% of the glycosomal PGK activity, the fraction that remains in the pellet after osmotic shock treatment of purified organelles, in contrast to the 77% soluble activity that is mainly attributed to PGKC. Antibodies against the unique 80 amino-acid insertion of PGKA blocked almost completely the glucose consumption by epimastigotes that were partially permeabilized with digitonin. These results indicate that PGKA is the predominant isoenzyme for sustaining glycolysis through the glycosomes of these parasites.

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26 1. Introduction, results and discussion

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27 In most trypanosomatids analyzed, phosphoglycerate kinase 28 (PGK) is present as three isoenzymes with different subcellular 29 locations (glycosomal PGK A and C; cytosolic PGKB) and molecular 30 weights. The three PGK isoenzymes of *Trypanosoma cruzi* are simul- 31 taneously expressed in each of its three developmental stages: 32 amastigotes, trypomastigotes and epimastigotes [1,2]. The glycoso- 33 mal isoenzymes A and C together were reported to be responsible 34 for approximately 20% of the total cellular PGK activity detected 35 in epimastigotes [1]. The presence of the three PGK isoenzymes 36 in different compartments and their simultaneous expression, do 37 raise the question about the role of each isoenzyme in the inter- 38 mediary metabolism of this parasite. Based on studies of *T. cruzi* 39 epimastigotes, we postulated previously that the principal role of 40 PGKA is its participation in the glycolytic pathway, when a high 41 ATP/ADP ratio within the glycosomes inhibits the PGKC activity [2].

42 Owing to a unique 80 amino-acid insertion in its N-terminal 43 domain, PGKA is the largest PGK isoenzyme in kinetoplastids 44 with a molecular weight of 56 kDa. The presence of a PGKA has 45 been reported in *Crithidia fasciculata*, *Trypanosoma brucei*, *Try- 46 panosoma congolense* and *T. cruzi*, but it could not be detected 47 in *Leishmania* species [1,3–6]. Previous studies strongly suggested 48 that the PGKA isoenzyme is tightly associated with the glycoso- 49 mal membrane in epimastigotes of *T. cruzi* [1]. Confirmation of 50 such association is now provided by the experiments presented 51 in Fig. 1. When epimastigotes were treated with increasing con- 52 centrations of the detergent digitonin, PGK activity was released 53 from the trypanosomes in two phases: 70–80% from the cyto- 54 sol at detergent concentrations less than approximately 0.05 mg 55 digitonin mg protein^{-1}, attributed to PGKB, and 20–30% between 56 approximately 0.04 and 0.20 mg digitonin mg protein^{-1}, mainly 57 attributed to PGKC and a minor part to PGKA [1,2], as shown in 58 Fig. 1A and B. However, the western blot of panel B, probed with a 59 polyclonal antiserum specific for the PGKA insert (α-PGKA pep- 60 tide antiserum) shows that most of the signal corresponding to 61 this isoenzyme is not released by this digitonin treatment, but 62 remains in the insoluble cell fraction, even in the presence of 0.1%

Abbreviations: ALAT, alanine aminotransferase; HK, hexokinase; ISDH, isocitrate dehydrogenase; PBS, phosphate-buffered saline; PGK, phosphoglycerate kinase.

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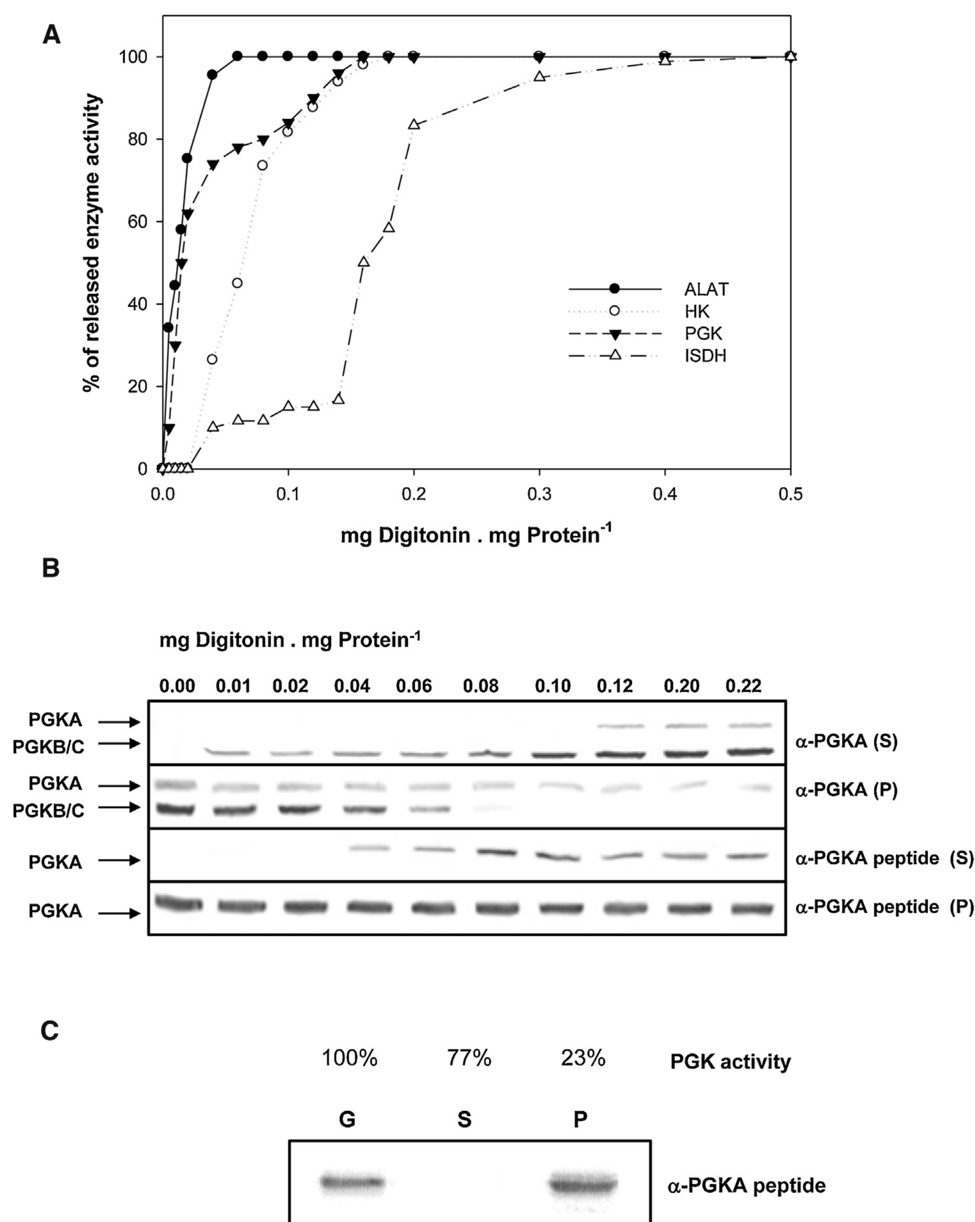

Fig. 1. (A) Release of PGK and marker enzymes from *T. cruzi* epimastigotes after treatment of the cells with digitonin. Epimastigotes of *T. cruzi* strain EP were axenically cultured at 28 °C, with gentle shaking, in monophasic LIT medium [10] supplemented with 10% heat-inactivated fetal bovine serum. Parasites were washed and suspended in a 20 mM Tris–HCl buffer, pH 7.2, containing 225 mM sucrose, 20 mM KCl, 10 mM KH_2PO_4, 5 mM $MgCl_2$ and 1 mM Na_2EDTA, at a concentration of 1 mg ml^{-1} protein and then aliquoted in several tubes. Increasing amounts of digitonin were successively added to each of these tubes, and the suspensions were incubated at room temperature for 20 min before being centrifuged at 7000 × *g* (Eppendorf centrifuge) for 2 min. Pellets were washed and resuspended in 200 μl of the same buffer. Enzymatic activities were assayed in the supernatants and, to establish the 100% release level, in cell suspensions in the presence of 0.1% (v/v) Triton X-100 and 150 mM NaCl that fully permeabilizes all cellular membranes. The activities were determined, as described previously [1,11,12], in the supernatants obtained after incubating the parasites at the indicated digitonin concentrations. Marker enzymes used were: for the cytosol, alanine aminotransferase (ALAT), glycosomes, hexokinase (HK) and mitochondrion, isocitrate dehydrogenase (ISDH). (B) The presence of the PGK isoenzymes as probed on western blots prepared of the supernatant (S) and pellet (P) fractions of the experiment of panel A. The polyclonal antisera used were the α-PGKA antiserum raised against full-length PGKA that recognizes all three isoenzymes and the α-PGKA peptide antiserum specific for this isoenzyme's insertion [2]. Note that PGKB and PGKC cannot be distinguished in western blots because of their similar molecular weight of 47 kDa [1,2]. (C) A preparation of highly purified glycosomes [13], equivalent to 1 mg of protein, was diluted 100 times with cold water and incubated at 4 °C for 30 min before being centrifuged at 105,000 × *g* for 90 min. The membranous fraction (pellet) from the glycosomes was resuspended in a minimal volume of buffer C (10 mM Tris–HCl, pH 7.4, 150 mM NaCl). The presence of PGKA in the supernatant (S) (glycosomal matrix) and pellet (P), as well as intact purified glycosomes (G) was assessed by western blotting using the polyclonal α-PGKA peptide antiserum [2]. The PGK activity of each of the fractions was also assayed.

Triton X-100. This affirms the notion of a strong association of the PGKA isoenzyme to the glycosomal membrane. Similar results using a different approach were obtained when fractions obtained upon osmotic shock treatment of purified glycosomes were analyzed by western blotting also using the specific α-PGKA peptide antiserum as a probe. The PGKA was only visualized in the pellet fraction (Fig. 1C), where also approximately 23% of the PGK activity was measured, while 77% of the glycosomal PGK activity was retrieved in the soluble fraction. The predominant soluble activity of the organelles should thus be attributed to the PGKC isoenzyme [2]. Importantly, this experiment demonstrates that the membrane-associated PGKA is active and, with the glycosomes responsible for 20% of the cellular PGK activity, accounts for approximately 6% of the total PGK activity of the cell. However, this membrane-associated PGKA activity was not detected in the release experiment of Fig. 1A.

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