Subcellular location of glycolytic enzymes in *Trypanosoma brucei* culture form

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Summary. The presence of a glycolytic complex or particle has been demonstrated in the insect midgut form of *Trypanosoma brucei brucei.* It differs from the 'glycosome' of the bloodstream form of the parasite in that 3-phosphoglycerate kinase is absent. The latter enzyme appears to be cytosolic.

The long, slender bloodstream form of *Trypanosoma brucei* has no electron transport-coupled phosphorylation[2] and depends entirely upon glycolysis for energy production. Under aerobic conditions, pyruvate is the resulting product, and reduced co-enzyme is re-oxidised by O_2 via the glycerol-3-phosphate-dihydroxyacetone phosphate shuttle which involves glycerol-3-phosphate oxidase and glycerol-3-phosphate dehydrogenase[3]. The former has been located in the pro-mitochondrion[4], and the latter is thought to be contained in a microbody-like particle, the 'glycosome', along with 8 other glycolytic enzymes[5-7]. As judged by enzyme content, there should be no net oxidation/reduction of coenzyme within the glycosome, and no net synthesis/consumption of ATP. The glycosome should catalyse the transformation of glucose to 3-phosphoglycerate under aerobic conditions, and to 3-phosphoglycerate and glycerol when glycerol-3-phosphate oxidase is inhibited by salicylhydroxamic acid, thus simulating anaerobiosis. Glycerol is thought to be formed by reversal of the glycerokinase reaction, favoured by the accumulation of glycerol-3-phosphate within the glycosome compartment[8] and rapid exit of glycerol from the cell[9].

We have undertaken to study the subcellular location of glycolytic enzymes in the insect midgut form of *T. brucei,* cultivated on a semi-defined medium. This form of the parasite has a well-developed mitochondrion with a complete respiratory function, and can use amino acids, such as proline, for ATP production[10]. These metabolic differences could possibly be reflected in the subcellular compartmentation of the glycolytic enzymes.

Material and methods. Strain STIB 366 was used in all experiments and cultivated in plastic tissue culture flasks at 27 °C on medium SDM-79[11]. The cells were harvested when they reached approximately 3.5 10^7 cells/ml.

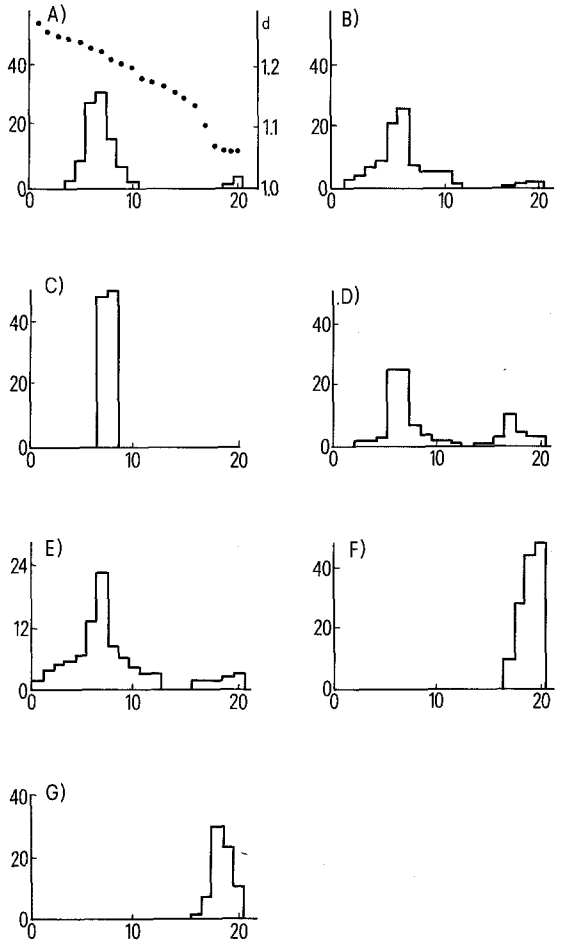
Cell rupture was performed in the following manner: 30 ml culture were centrifuged at 1000 × g for 10 min, washed with Krebs-Ringer saline solution, containing 0.025 M tris-maleate pH = 7.3, and resuspended in 0.5–2.0 ml buffer containing the following additives: 50 mM dithiothreitol, 0.1 mM phenylmethanesulfonyl fluoride, 0.1 mM EDTA. 3–6 vol. bi-distilled water, containing these same additives, were added slowly to allow maximum swelling without rupture. The swollen cells were broken by nitrogen cavitation (pressure: 55 bar), after which 3 M KCl was added to a final concentration of 0.15 M. The resulting homogenate was used in differential centrifugations or mixed with an equal weight of 55% (w/w) sucrose in buffer for layering onto sucrose density gradients.

Isopycnic centrifugations were performed on 28–56% sucrose gradients in buffer at 4 °C. Approximately 2.5 ml sucrose-containing homogenate was layered on to each 10 ml gradient. The gradients were centrifuged for 3 h at 208,000 × g (bottom of tube) in a SW41 Beckman rotor.

Enzyme assays were performed according to Bergmeyer[12] with the following modifications: in the hexokinase assay, the ATP concentration was 1.33 mM, and in the glyceraldehyde phosphate dehydrogenase assay the 3-phosphoglycerate kinase concentration was 80 µg/ml. All enzymes were purchased from Boehringer-Mannheim.

Permeabilization of whole cells was carried out as follows: 15 ml *T. brucei* culture were centrifuged at 1000 × g for 10 min and washed once in buffer. The cells were resuspended in 2.0 ml buffer and incubated at room temperature for 30 min with 2 µl nystatin solution (50 mg/ml dimethylsulfoxide). The permeabilized cells were centrifuged for 10 min at 1000 × g and enzyme activities were determined in the pellet and in the re-centrifuged supernatant.

Results. We have investigated the subcellular location of hexokinase, triosephosphate isomerase, glycerol-3-phosphate dehydrogenase, glyceraldehyde phosphate dehy-



Distribution of 7 glycolytic enzymes on a 28–56% sucrose gradient. The activity in each fraction is expressed as a percentage of total recovered activity (ordinate). Fraction numbers are given on the abscissa. The dotted line shows the density of the fractions, according to the scale to the right of graph A.

A hexokinase; *B* triosephosphate isomerase; *C* glycerol-3-phosphate dehydrogenase; *D* glyceraldehyde phosphate dehydrogenase; *E* glycerolkinase; *F* pyruvate kinase; *G* 3-phosphoglycerate kinase.

drogenase, 3-phosphoglycerate kinase, glycerokinase and pyruvate kinase. Of these enzymes, all but pyruvate kinase are particulate in the bloodstream form of *T. brucei*[6].

Table 1 shows the distribution of these enzymes between the washed pellet and the supernatant, obtained by centrifugation of a homogenate for 15 min at 14,500 × g. It also shows the percentage of latency of these enzymes in each fraction (percentage of total measured activity which is liberated by Triton X-100). All enzymes exhibit some degree of latency, although latency is comparatively small for pyruvate kinase and 3-phosphoglycerate kinase. Both of these enzymes are found mainly in the supernatant fraction, whereas all the others are found mainly in the pellet.

Furthermore, when 3 successive 10-min centrifugations were performed at 1000 × g, 10,000 × g and 14,500 × g, 47%, total hexokinase activity was found in the 14,500 × g pellet, which contained only 7% total protein. The 1000 × g and 10,000 × g pellets, and the final supernatant contained respectively 20, 27, and 6% hexokinase activity and 21, 8 and 64% total protein.

The figure shows the distribution of the above-mentioned enzymes on a 28–56% sucrose density gradient. Whereas pyruvate kinase and 3-phosphoglycerate kinase remain at the top of the gradient, the remaining enzymes form a band with maximum activity at 1.225 g/cm^3, leaving only a small percentage, probably due to broken complexes or particles, at the top.

These results are very similar to those obtained in the bloodstream form of the parasite[6], with one notable exception: 3-phosphoglycerate kinase, which is particulate in the bloodstream form, appears to be cytoplasmic in our experiments. In order to determine whether this result was due to our procedure of cell rupture, *T. brucei* culture form cells were 'permeabilized' by the antibiotic nystatin which is known to damage the plasma membrane of fungi by interaction with sterols[13]. Under our conditions, *T. brucei* culture form cells swelled and became round upon addition of nystatin. Hexokinase, glyceraldehyde phosphate dehydrogenase and 3-phosphoglycerate kinase were assayed in the centrifuged permeabilized cells and in the supernatant of permeabilization, in the presence and in the absence of 0.1% Triton X-100. The results are presented in table 2. It appears that the permeabilization procedure causes 3-phosphoglycerate kinase to leak from the cells, whereas hexokinase remains within. Furthermore, 44% of the 3-phosphoglycerate kinase activity which remains in the pellet can be measured without addition of Triton, that is, without total disruption of the plasma membrane. We conclude that 3-phosphoglycerate kinase is not a part of the glycosome or glycolytic complex of insect midgut form *T. brucei*.

Discussion. We have produced evidence for the existence of a glycolytic complex or particle in the insect midgut form of *T. brucei*. Furthermore, we have shown that the enzyme content of this complex or particle differs from that of the 'glycosome' of the bloodstream form. What is the consequence of this change in location on the activity of the particle, and on glycolytic ATP production? The bloodstream form has no other source of energy than glycolysis and degrades glucose very rapidly. The culture form, on the other hand, has practically no need of glycolytic ATP and the actual importance of glycolysis in the organism's metabolism is unknown. An understanding of what happens to the 'glycosome' when 3-phosphoglycerate kinase is excluded or detached could provide an answer. Another question which arises is whether or not the 'glycosome' is indeed a membrane-surrounded particle. If so, it would be interesting to understand the mechanism which determines whether or not 3-phosphoglycerate kinase will be included in the particle, and the mechanism of ATP permeation which becomes necessary if glycolysis is to proceed in the culture form. Also, since the salicylhydroxamic acid-sensitive glycerophosphate oxidase does not contribute to respiration in the culture form of the strain used, it is unknown how the balance of oxidating/reducing equivalents is maintained within the 'glycosome'.

Regardless of whether or not the glycolytic particle is membrane-surrounded, the observation that a glycolytic enzyme association exists, and that it differs in these 2 forms of *T. brucei*, is certainly important for understanding the physiology of the parasite.

Table 1. Distribution of glycolytic enzymes between the washed 14,500 × g pellet and the corresponding supernatant (L = latency). The activities are expressed as a percentage of total activity in the homogenate

Enzyme	Enzyme activity Pellet	Supernatant
Hexokinase	80 (L = 80%)	1.6 (L = 0%)
Triosephosphate isomerase	118 (L = 74%)	3.7 (L = 60%)
Glyceraldehyde phosphate dehydrogenase	77 (L = 100%)	24 (L = 0%)
Phosphoglycerate kinase	1	99 (L = 5%)
Glycerol-3-phosphate dehydrogenase	51 (L = 100%)	0
Glycerolkinase	68 (L = 85%)	7.5 (L = 80%)
Pyruvate kinase	2.5 (L = 50%)	97.5 (L = 65%)

Table 2. Distribution of hexokinase and 3-phosphoglycerate kinase between the pellet of permeabilized cells and the supernatant of permeabilization, in the presence and in the absence of 0.1% Triton X-100. The activities are expressed as a percentage of the total activity, liberated by Triton X-100 from an identical aliquot of non-permeabilized cells

Enzyme	Total activity (%) Pellet	Pellet + Triton	Supernatant	Supernatant + Triton
Hexokinase	0	98.4	0	0
3-Phosphoglycerate kinase	21.3	47	51	51

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