

LOCALIZATION OF NINE GLYCOLYTIC ENZYMES IN A MICROBODY-LIKE ORGANELLE IN *TRYPANOSOMA BRUCEI*: THE GLYCOSOME

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1. Introduction

The African trypanosomes that belong to the sub-genus *Trypanozoon* are completely dependent on glycolysis for energy production in the bloodstream of the vertebrate host, because the biosynthesis of respiratory chain and Krebs cycle enzymes is repressed (see ref. [1]). The glycolytic pathway is modified in two respects: glucose is converted into pyruvate rather than lactate and the NADH produced in glycolysis is reoxidized indirectly by O₂ via the coupled action of NAD⁺-linked glycerol-3-phosphate dehydrogenase and glycerol-3-phosphate oxidase [2].

We have shown previously that in *Trypanosoma brucei* the oxidase is located in the repressed promitochondrion and that the dehydrogenase co-purifies with microbodies [3,4]. The latter finding raised the question whether other glycolytic enzymes might be particle-bound as well. We have already briefly reported that this is indeed so for glyceraldehyde-phosphate dehydrogenase and phosphoglycerate kinase [4,5]. In this paper we show that glycerol kinase and seven glycolytic enzymes involved in converting glucose into 3-phosphoglycerate are located in a microbody-like organelle as well.

2. Materials and methods

Trypanosoma brucei EATRO 427 was grown in

Abbreviations: DHAP, dihydroxyacetonephosphate; G-3-P, glycerol-3-phosphate; 3-PGA, 3-phosphoglycerate; P_i, inorganic phosphate

rats and isolated as described previously [6,7]. Purified trypanosomes (2.2 g wet weight) were homogenized and homogenates fractionated into a nuclear fraction, a large-granule (mitochondria and microbody enriched) fraction, a small-granule fraction and the final supernatant as described before [3]. For isopycnic centrifugation 0.5 ml of a large-granule fraction (5.5 mg protein) was layered on top of a sucrose gradient, linear from 1.18–2.0 M sucrose ($\rho = 1.15\text{--}1.26\text{ g/cm}^3$) and supplemented with a cushion of 2.5 M sucrose. The gradient was centrifuged in a SW-41 rotor in a Beckman L2-65B ultracentrifuge at 30 000 rev./min for 17 h at 6°C. Fractions were collected from the bottom of the tube as described previously [4] and stored in liquid nitrogen. For all enzyme determinations, except glycerol-3-phosphate oxidase [3], fractions were diluted 4-fold with 50 mM phosphate buffer (pH 7.2) containing 0.25% Triton X-100.

Hexokinase (EC 2.7.1.1) [8], glucosephosphate isomerase (EC 5.3.1.9) [8], 6-phosphofructokinase (EC 2.7.1.11) [8], aldolase (EC 4.1.2.13) [8], triose-phosphate isomerase (EC 5.3.1.1) [8], glyceraldehydephosphate dehydrogenase (EC 1.2.1.12) [9], phosphoglycerate kinase (EC 2.7.2.3) [10], enolase (EC 4.2.1.11) [8], glycerol kinase (EC 2.7.1.30) [11] and alanine aminotransferase (EC 2.6.1.2) [8] were assayed according to described procedures. NAD⁺-linked glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) was measured with 1 mM DHAP as substrate essentially as described previously [3]. Protein was determined by the method of Lowry et al. [12] with bovine serum albumin as standard.

For the calculation and presentation of the results

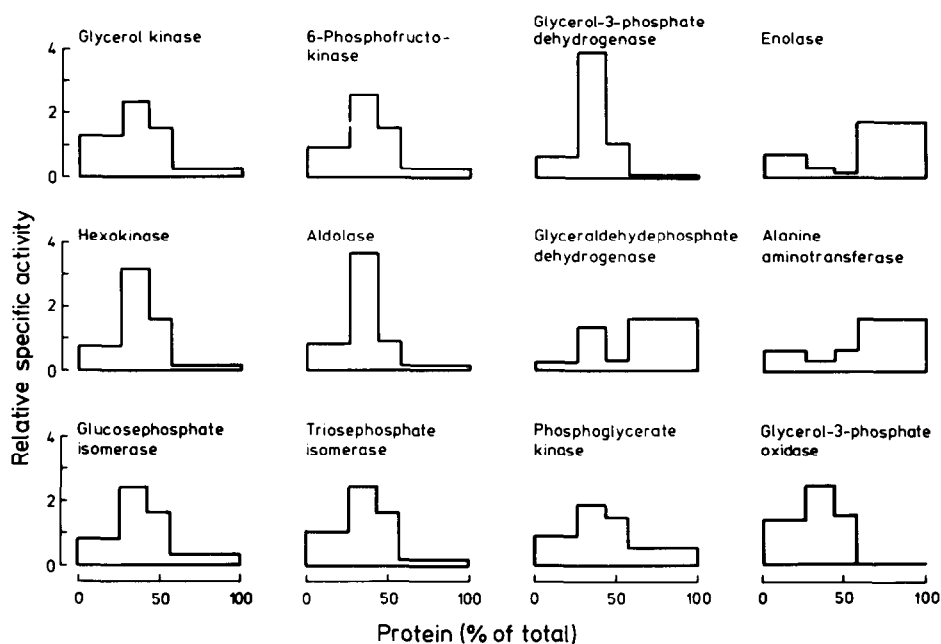


Fig.1. Distribution of *T. brucei* enzymes in fractions obtained by differential centrifugation. Fractions are plotted in the order of their isolation, i.e. (from left to right) nuclear, large-granule, microsomal and supernatant fractions. The ordinate represents the relative specific activity (percentage of total activity/percentage of total protein). The abscissa presents the cumulative protein content.

Table 1
Fractionation of *T. brucei* by differential centrifugation

Enzyme	Absolute value in cytoplasmic extract <i>plus</i> nuclear fraction (mg protein or U*/mg protein)	Distributions in fractions (% of recovery)				Recovery (%)
		N	M	P	S	
Protein	144	26	16	15	42	119
Glycerol-3-phosphate oxidase	0.18	37	39	23	1	78
Hexokinase	0.79	20	50	23	7	95
Phosphoglucose isomerase	0.54	21	39	25	15	90
6-Phosphofructokinase	1.63	25	41	23	11	100
Aldolase	0.36	22	59	13	6	81
Triosephosphate isomerase	1.18	28	38	25	9	77
Glycerol-3-phosphate dehydr.	0.73	18	63	16	3	125
Glycerol kinase	2.60	30	37	23	10	77
Glyceraldehydephosphate dehydr.	0.05	6	21	5	68	131
Phosphoglycerate kinase	0.43	24	30	23	23	89
Enolase	0.08	19	5	2	74	79
Alanine aminotransferase	0.53	16	6	10	68	62

* Units expressed as $\mu\text{mol} \times \text{min}^{-1} \times \text{mg protein}^{-1}$

of differential and isopycnic centrifugation the methods described by De Duve as reviewed by Beaufay and Amar-Costesec [13] were used.

All enzymes, coenzymes and substrates were from Boehringer GmbH, Mannheim.

3. Results

A *T. brucei* homogenate was fractionated by differential centrifugation into a nuclear (N), a large-granule (M), a microsomal (P) and a cell-sap (S) fraction [3]. The distribution of protein, the mitochondrial marker glycerol-3-phosphate oxidase [4], the microbody marker NAD⁺-linked glycerol-3-phosphate dehydrogenase [4] and the cell-sap marker alanine aminotransferase was determined and compared with the distribution of glycerol kinase and seven glycolytic enzymes. The results are presented in fig.1 and table 1.

Most glycolytic enzymes and glycerol kinase are present in high activity, well above the known rate of glycolysis in *T. brucei* of 0.15–0.2 $\mu\text{mol}/\text{min}/\text{mg}$ protein. These enzymes are for 77–94% particle-bound and distribute rather like NAD⁺-linked glycerol-3-phosphate dehydrogenase. Low activities were found for glyceraldehydephosphate dehydrogenase and enolase and we attribute this to inactivation of the enzymes during storage. With the former enzyme there is substantial activity in the large-granule fraction; the relatively high activity in the cell-sap fraction could be due to leakage of enzyme from the particles, as observed for catalase with the insect trypanosome *Crithidia luciliae* [4]. With enolase very little activity is found in the M and P fractions and this is probably a cell-sap enzyme.

To test whether the glycolytic enzymes are associated with promitochondria or other organelles a large-granule fraction was centrifuged to equilibrium in a sucrose gradient, designed to give optimal resolution in the high-density region. Figure 2 shows that the pro-mitochondria (glycerol-3-phosphate oxidase) form a broad band in the upper half of the gradient, completely separated from the microbody marker enzyme NAD⁺-linked glycerol-3-phosphate dehydrogenase, which bands at about 1.23 g/cm³ with the bulk of the protein (mainly flagella and microbodies [4]). It is clear that the glycolytic enzyme activities band with the microbody marker enzyme.

We have shown previously that NAD⁺-linked glycerol-3-phosphate dehydrogenase shows latency and can be activated, in part by freezing and thawing and completely with Triton X-100 [3,4]. Similar 5–10-fold activation by Triton was found for the particulate activity of the other glycolytic enzymes tested with the exception of enolase (results not shown). This supports the concept that these enzymes are present within a microbody-like organelle rather than adsorbed to particles.

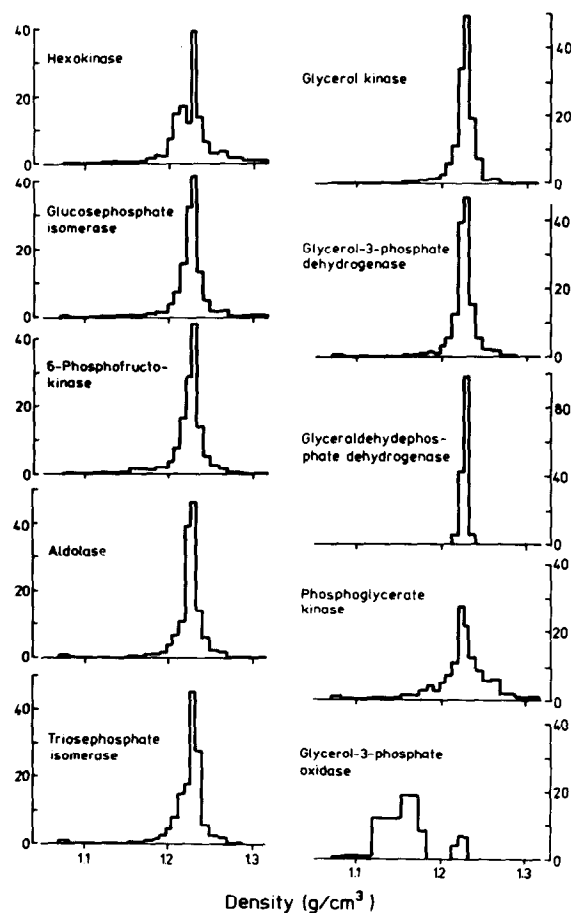
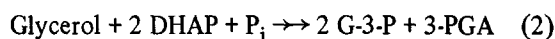
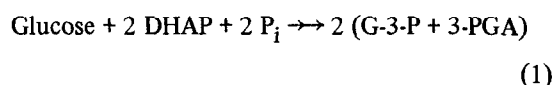


Fig.2. Isopycnic centrifugation in sucrose of a large-granule fraction (5.5 mg protein) from *T. brucei*. The percentages of recovery are 121 for hexokinase, 187 for glucosephosphate isomerase, 108 for 6-phosphofructokinase, 157 for aldolase, 79 for glycerol kinase, 114 for glycerol-3-phosphate dehydrogenase, 67 for glyceraldehydephosphate dehydrogenase, 153 for phosphoglycerate kinase and 119 for glycerol-3-phosphate oxidase. The recovery of triosephosphate isomerase was not determined.

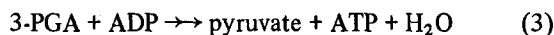
4. Discussion

Our results show that a major part of glycolysis in bloodstream *T. brucei* is present in an organelle, tentatively identified as a microbody in earlier experiments [3,4]. We assume that all particle-bound glycolytic enzymes and glycerol kinase are present in the same organelle and that the activity of these enzymes found in the cell-sap fraction is due to damage of particles. Because the organelle lacks typical microbody or peroxisomal enzymes [3,4], but contains a major part of the glycolytic pathway, we provisionally call it a 'glycosome'.

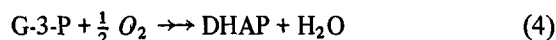
From its enzyme content the glycosome should be able to catalyze the following reactions:



In both overall reactions ATP utilization is balanced by ATP synthesis and NAD^+ reduction by NADH oxidation. We assume that 3-PGA (or 2-PGA) leaves the glycosome and is converted to pyruvate in the cell sap with concomitant net glycolytic ATP synthesis:



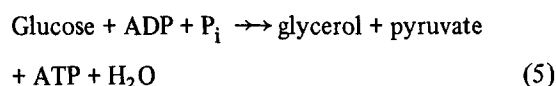
The other product of glycosome metabolism, G-3-P, is oxidized in the pro-mitochondrion:



It is clear, therefore, that DHAP, G-3-P and 3-PGA should be able to rapidly pass the glycosomal membrane. On the other hand, all glycosomal enzymes are latent, suggesting a permeability barrier for phosphorylated substrates and co-factors. We, therefore, propose that the glycosomal membrane is only readily permeable to glucose and glycerol and impermeable to NAD, adenine nucleotides and glycolytic intermediates. The triosephosphates and P_i should pass the membrane by means of specific translocators, e.g., a translocator exchanging G-3-P and DHAP, one exchanging 3-PGA and P_i and possibly one exchanging P_i and OH^- . In this concept the glyco-

some has evolved to optimize conditions for glycolysis by creating a compartment where high and optimal concentrations of enzymes, substrates and co-factors are maintained. The advantage of such compartmentation for catalytic efficiency has been predicted on theoretical grounds [14,15].

The location of glycolytic enzymes in the glycosome also provides a clue to the mechanism of anaerobic glycolysis in *T. brucei*. When reaction 4 is inhibited by anaerobiosis (see ref. [1]) or salicylhydroxamic acid [16], glucose is converted into glycerol + pyruvate. We have previously obtained evidence [7,16] that this reaction is coupled to net ATP synthesis:



Several reaction schemes have been proposed [7,16–18] to account for the stoichiometry of reaction (5), but none of these has yet been confirmed by experiments. There is no significant G-3-Pase activity ($0.02 \mu\text{mol} \times \text{min}^{-1} \times \text{mg protein}^{-1}$, Oppendoes, F. R. and Bloemen, L. J. N. G. M., unpublished) or glycerol dehydrogenase activity [16,17] detectable. The glycosome concept provides an additional explanation for reaction (5), however. Anaerobiosis blocks reaction (4) and leads to a rapid depletion of DHAP in the cell sap. This stops the exchange of G-3-P and DHAP through the glycosomal membrane, resulting in an accumulation of G-3-P within the glycosome. We have, in fact, observed a large increase in G-3-P when reaction (4) is inhibited with salicylhydroxamic acid (Oppendoes, F. R. and Bloemen, L. J. N. G. M., unpublished) and if this G-3-P would be confined to the glycosome, local concentrations of 0.1 M might be reached. This could trigger the switch to anaerobic glycolysis utilizing one of the pathways previously postulated [7,16–18], but more interestingly, it might even lead to the formation of glycerol and ATP by a reversal of the glycerol kinase reaction. This novel explanation for reaction (5) only requires known trypanosomal enzymes and it would also explain the presence of an exceedingly high glycerol kinase activity (see table 1) in the highly specialized glycosome, even though glycerol is not a readily available substrate in

the blood of the vertebrate host. Experiments are in progress to test this hypothesis.

Acknowledgements

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