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Structure, Function, and Biogenesis of Glycosomes in Kinetoplastida

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Glycosomes are intracellular, microbody-like organelles found in all members of the protist order Kinetoplastida examined. Nine enzymes involved in glucose and glycerol metabolism are associated with these organelles. These enzymes are involved in pathways which, in other organisms, are usually located in the cytosol. This paper reviews our current knowledge about the glycosome and its constituent enzymes, with special reference to the organelle of *Trypanosoma brucei.*

KEY WORDS: Glycosome; microbody; glycalysis; biogenesis.

GLYCOSOMES IN *TRYPANOSOMA BRUCEI*

In trypanosomes a unique form of metabolic compartmentalization is found: most of the enzymes of the glycolytic pathway are contained within a microbody-like organelle, called glycosome. The research on this organelle and its constituent enzymes has been carried out mainly on the African trypanosome *Trypanosoma brucei.* However, glycosomes have been detected in all members of the protist order Kinetoplastida examined: in representatives of both the Trypanosomatina (*Trypanosoma, Leishmania, Crithidia, Phytomonas*) and the Bodonina (*Trypanoplasma*). This subcellular compartmentation of glycolysis in trypanosomes is fundamentally different from the situation in all other eukaryotic organisms studied, where glycolysis occurs in the cytosol.

The glycosomes in *T. brucei* contain nine enzymes involved in, or related to, glycolysis, which can account for the conversion of glucose to 3-phosphoglycerate plus glycerol (Fig. 1) (Opperdoes and Borst, 1977; Visser and Opperdoes, 1980). In addition to glycolytic enzymes, these organelles also contain part of the adenylate kinase activity (Opperdoes *et al.*, 1981), the last two enzymes of pyrimidine biosynthesis (Hammond *et al.*, 1981), enzymes involved in the synthesis of ether lipids (Opperdoes, 1984), and, in cultured procyclics, malate dehydrogenase (MDH)[2] (Opperdoes *et al.*, 1981) and phospho*enol*pyruvate carboxykinase (PEPCK) (Opperdoes and Cottem, 1982).

The enzymatic content of the glycosomes varies during the life cycle of the trypanosome (Hart *et al.*, 1984). In *T. brucei* cells living in the mammalian bloodstream, the role of the organelle is mainly glycolysis: the nine enzymes involved in the conversion of glucose into phosphoglycerate and, under anaerobiosis, glycerol constitute more than 90% of the organelle's protein content. In cultured trypanosomes, representative of the procyclic trypomastigote living in the midgut of the tsetse fly, the level of the majority of the glycosomal enzymes involved in glycolysis is decreased. For other proteins, notably PEPCK and MDH, a significantly higher activity

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[2] ALD: aldolase; DHAP: dihydroxyacetonephosphate; cGADPH: cytosolic glyceraldehydephosphate dehydrogenase; gGAPDH: glycosomal glyceraldehydephosphate dehydrogenase; GDH: glycerolphosphate dehydrogenase; GK: glycerol kinase; G-3-P: glycerol-3-phosphate; HK: hexokinase; MDH: malate dehydrogenase; PEPCK: phospho*enol*pyruvate carboxykinase; PGI: glucosephosphate isomerase; cPGK: cytosolic phosphoglycerate kinase; gPGK: glycosomal phosphoglycerate kinase; PYK: pyruvate kinase; TIM: triosephosphate isomerase.

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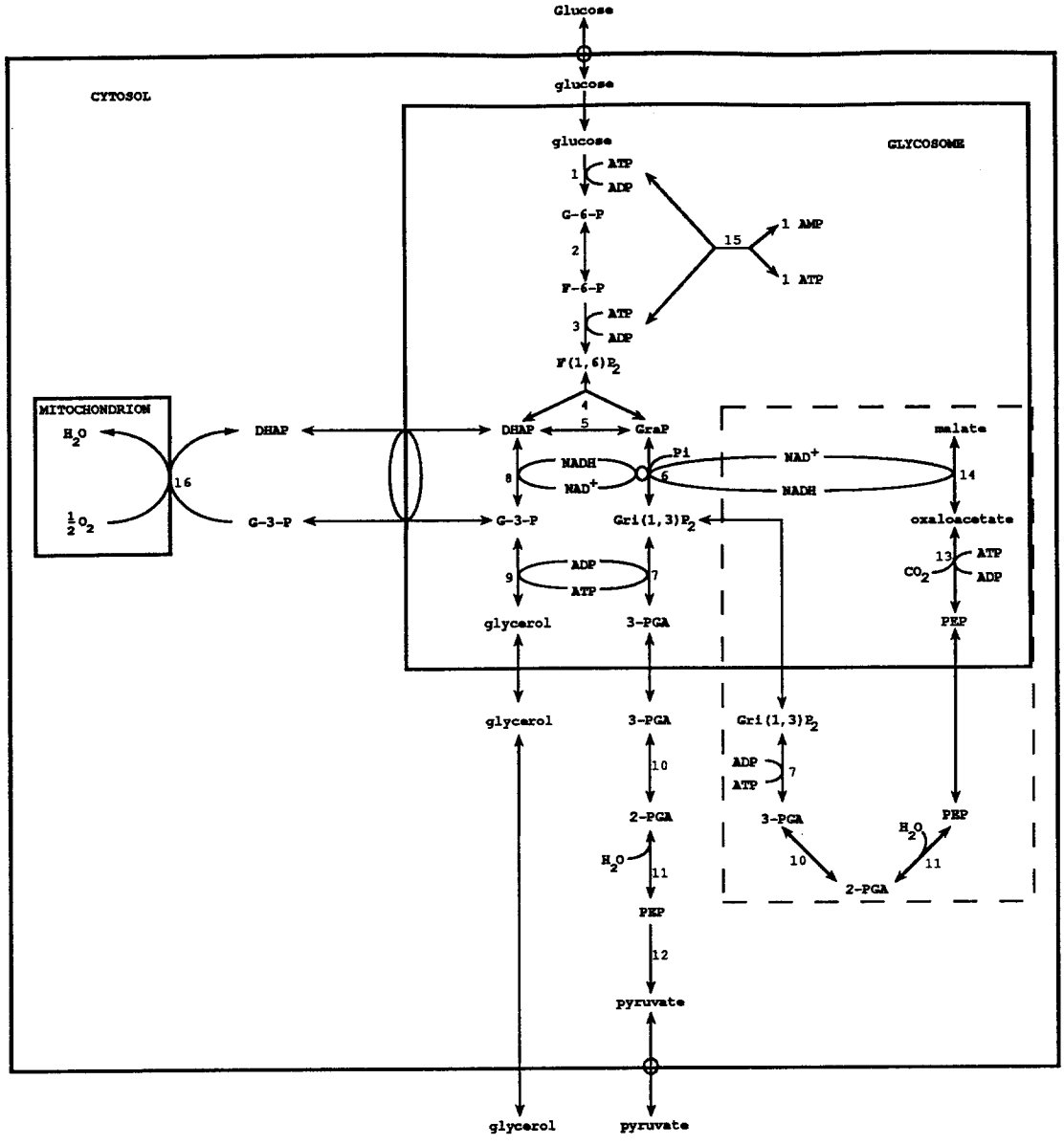

Fig. 1. Compartmentalization of glycolysis in *T. brucei*. Reactions located in the dashed box occur only in procyclic trypomastigotes. 1. hexokinase; 2. glucosephosphate isomerase; 3. phosphofructokinase; 4. aldolase; 5. triosephosphate isomerase; 6. glyceraldehyde-3-phosphate dehydrogenase; 7. phosphoglycerate kinase; 8. glycerol-3-phosphate dehydrogenase; 9. glycerol kinase; 10. phosphoglycerate mutase; 11. enolase; 12. pyruvate kinase; 13. phospho*enol*pyruvate carboxykinase; 14. malate dehydrogenase; 15. adenylate kinase; 16. glycerol-3-phosphate oxidase.

can be detected. These variations in the composition of the glycosome tally with the changes in the overall metabolic activity of the parasite. Bloodstream form trypanosomes are for their energy supply under aerobic conditions entirely dependent on the conversion of glycose into pyruvate. They lack a functional Krebs cycle and the mitochondrial system for oxidative phosphorylation. Pyruvate is the sole end product, and is excreted into the host's bloodstream. The procyclic forms rely more on mitochondrial activity. Other substrates than glucose, such as amino acids and fatty acids, are also used. Acetate and succinate are excreted rather than pyruvate.

In the bloodstream form of *T. brucei*, the glycolytic

pathway is organized in such a way that the enzymes for conversion of glucose into phosphoglycerate are inside the glycosome, while those catalyzing the last part of the pathway are localized in the cytosol. As a consequence, net ATP synthesis occurs in the cytosol, whereas the consumption and production of ATP are balanced within the organelle (see Fig. 1). Similarly, no net change in the redox state of NAD takes place in the glycosome because, during aerobic glycolysis, the NADH produced is reoxidized via the G-3-P/DHAP shuttle, which couples the glycolytic pathway to a mitochondrial glycerolphosphate oxidase. This respiratory process is not linked to ATP synthesis. When the oxidase is unable to operate, e.g., under anaerobic conditions or in the presence of its inhibitor salicylhydroxamic acid, glucose is converted into equimolar amounts of pyruvate and glycerol which are excreted. It has been demonstrated that the production of glycerol can be coupled to the synthesis of ATP by reversal of the glycerol kinase reaction (Visser *et al.*, 1981). This is made possible by the low phosphate potential that prevails in the glycosome and the high concentration of glycerol-3-phosphate (Hammond *et al.*, 1985; Opperdoes, 1987). Therefore, under anaerobic conditions as well, no net change in ATP and NAD levels occurs in the glycosomal compartment during glucose metabolism. This is probably also the case in procyclic trypanosomes. Several of the glycolytic enzymes in the glycosome are considerably repressed during this stage of the life cycle. Glucose consumption in the insect stage is accompanied by the fixation of CO_2. This results in the excretion of significant amounts of succinate under aerobic as well as anaerobic conditions. The glycosomal CO_2 fixation via PEPCK, which accompanies the glucose consumption, serves to reoxidize the glycolytically produced NADH by MDH, because the G-3-P/DHAP shuttle is inoperative in procyclic cells (see Fig. 1).

From the discussion above it is clear that the glycosome is a morphologically and functionally distinct organelle within the trypanosome, capable of maintaining a phosphate potential and redox state that must be different from those prevailing in the cytosol. This suggests that the membrane surrounding the organelle is a true permeability barrier. This idea is supported by *in vivo* pulse-labelling experiments which demonstrated that the glycosomal pool of glycolytic intermediates equilibrates only slowly with the remainder of the cell (Visser *et al.*, 1981).

THE MORPHOLOGY AND ORGANIZATION OF THE GLYCOSOME

Microbodies are organelles present in all eukaryotic cells except the most primitive protists, sometimes referred to as Archezoa (Cavalier-Smith, 1987). Although the glycosome is an organelle with a unique enzymatic content, it has a number of features suggesting that it should be classified as a member of the microbody family, to which also peroxisomes and glyoxysomes belong (Opperdoes, 1988). First, glycosomes of Kinetoplastida strongly resemble in their morphology the microbodies of other eukaryotes. In *T. brucei* they constitute a single population of organelles, of extremely homogeneous size, with an average diameter of 0.27 μm. They appear in electron micrograph sections as round or oval-shaped, electron-dense bodies with a finely granular matrix, and are surrounded by a single membrane with a thickness similar to that of the membranes of endoplasmic reticulum and mitochondrion (6–7 nm) (Opperdoes *et al.*, 1984). Highly purified glycosomes from the bloodstream form contain predominantly two phospholipids: phosphatidyl choline and phosphatidyl ethanolamine in a ratio of approximately 2 : 1, and some sphingomyelin. Similar preparations from procyclic insect stages contain in addition some phosphatidyl serine and phosphatidyl inositol. The contribution of glycosomes to the total cell volume is noteworthy; together they represent 4.3% of the volume of the cell. Morphometric studies showed that each *T. brucei* cell contains on average 230 glycosomes. They represent 8–9% of the total cellular protein. The protein concentration of a *T. brucei* cell is 175 mg/ml, whereas the protein concentration in the glycosome is between 320 and 360 mg/ml. This value is higher than that of mammalian peroxisomes. Seventy percent of these proteins is present in the matrix, whereas the remainder is associated with the glycosomal membrane.

Furthermore, glycosomes contain some enzymes that are usually associated with microbodies and peroxisomes, such as those involved in β-oxidation and ether-lipid biosynthesis (Opperdoes, 1984, 1988). Catalase, a typical microbody enzyme, is not detectable in most representatives of the Kinetoplastida. However, when it is present, such as in *Crithidia luciliae*, *Phytomonas* sp., and *Trypanoplasma borelli*, it is located in the glycosome.

Another feature that the glycosome has in common with other microbodies is its route of

biosynthesis. Glycosomes, like other microbodies, do not contain any DNA. Thus, in contrast to the situation in mitochondria and chloroplasts, all their proteins are encoded on nuclear chromosomes, synthesized on free ribosomes in the cytosol, and imported post-translationally into the organelle without any detectable form of processing (Hart *et al.*, 1987; Clayton, 1987, 1988; Borst, 1989).

As in other microbody-like organelles, controversy surrounds their multiplication. Although it is widely believed that microbodies are formed by growth and division of pre-existing microbodies (Borst, 1989), direct evidence supporting this view is lacking, nor is anything known about what controls the shape, number, and position of these organelles during the cell cycle.

IMPORTANCE OF GLYCOSOMES FOR GLYCOLYSIS IN *T. BRUCEI*

Glycolysis is essential for African trypanosomes living in the bloodstream of the mammalian host, because it is their sole source of energy. They lack a functional Krebs cycle, a mitochondrial ATP-synthesizing respiratory chain, and significant polysaccharide reserves or high-energy phosphate stores such as creatinephosphate or polyphosphates. Therefore, they are entirely dependent on the aerobic conversion of glucose to pyruvate (Bowman and Flynn, 1976). This dependence on an inefficient energy provision is compensated by an extremely high glucose consumption. The glycolytic flux of carbon through the *T. brucei* cell is very high: 0.08 μmol glucose are consumed per min per mg protein (Opperdoes, 1987). It has been argued that the compartmentalization of glycolysis within an organelle allows this extremely high glycolytic flux by locally increasing the substrate concentration (Misset *et al.*, 1986; Opperdoes, 1987). The intracellular concentration of glycolytic intermediates in *T. brucei* is not dissimilar from that of other cells (Visser and Opperdoes, 1980). But glycosomes, representing only 4.3% of the cellular volume, contain 20–30% of the glycolytic intermediates. The concentration of these intermediates in the glycosomal compartment is then at least five times higher than that in the cytosol (Misset and Opperdoes, 1984; Misset *et al.*, 1986). These authors estimated the concentration of both active sites and metabolites in the glycosome for three steps of the glycolytic pathway. They concluded that these concentrations were similar and in the millimolar range. Since each of these enzymes has a K_m for its respective substrate of the same order of magnitude, a high proportion of the glycosomal metabolites must be protein-bound. High intraglycosomal metabolite and active-site concentrations may allow the trypanosome to sustain an extreme glycolytic flux with a relatively small amount of protein. The high glycolytic efficiency of the trypanosome is probably best illustrated by the fact that in muscle, which has a high demand for glucose, GAPDH can constitute over 10% of the soluble protein, while in trypanosomes this enzyme represents only 0.5% of the total. Nevertheless, the glycolytic rate of the African trypanosome is by far superior to that of any other eukaryotic cell type.

PROPERTIES OF GLYCOSOMAL ENZYMES IN *T. BRUCEI*

All nine glycosomal enzymes involved in glucose and glycerol metabolism of *T. brucei* have been purified to near-homogeneity, as well as two glycolytic enzymes which are also represented in the cytosol [glyceraldehydephosphate dehydrogenase (cGAPDH) and phosphoglycerate kinase (cPGK)], and pyruvate kinase (PYK) which is exclusively present in the cytosol (Misset *et al.*, 1986, 1987; Misset and Opperdoes, 1987; Callens *et al.*, 1991). Moreover, the genes coding for several glycolytic enzymes have been cloned and sequenced. These include those for the glycosomal enzymes glucosephosphate isomerase (PGI), aldolase (ALD), triosephosphate isomerase (TIM), gGAPDH, gPGK, and glycerol-3-phosphate dehydrogenase (GDH) (Marchand *et al.*, 1989; Clayton, 1985; Marchand *et al.*, 1988; Swinkels *et al.*, 1986; Michels *et al.*, 1986; Osinga *et al.*, 1985; Kohl *et al.*, 1994). In addition, the genes for the cytosolic enzymes cGAPDH, cPGK, and PYK were characterized (Michels *et al.*, 1991; Osinga *et al.*, 1985; Allert *et al.*, 1991). Two tandemly linked, completely identical genes have been found for the gGAPDH and ALD; PYK is encoded by two nearly identical (99%) genes, while for TIM, PGI, GDH, and cGAPDH only one gene is present. In the case of PGK three tandemly linked, but not identical, genes have been described, of which one (PGK-B) codes for the cytoplasmic enzyme, and another (PGK-A) for a minor glycosomal enzyme that has an internal 80-amino acids insertion relative to all other PGKs (Alexander and Parsons, 1991; Swinkels *et*

al., 1992). The third PGK (PGK-C) is the major glycosomal enzyme (Osinga *et al.*, 1985).

When the properties of the purified enzymes and their primary structures predicted from gene sequences were compared with each other and with the glycolytic enzymes of other organisms, two major features of the glycosomal proteins appeared. First, with the exception of TIM, each of the glycosomal enzymes of *T. brucei* has a relative molecular mass that is 1–5 kDa larger than that of the homologous cytosolic enzymes examined from whatever other organism. This is due to the presence of specific insertions or extensions at either the N- or C-terminus of the proteins. Second, all glycosomal enzymes have a high isoelectric point (pI 8.7–10.0), except PGI (pI 7.5). These values are higher than those of the cytosolic enzymes cGAPDH and cPGK (pI 7.9 and 6.2, respectively) and higher than those reported for the corresponding enzymes of any other organism. The high pI's are due to the presence of additional positively charged amino acids in the glycosomal proteins. They could play a role in neutralizing the negative charges of the phosphorylated glycolytic metabolites present in large quantities inside the glycosome (Misset *et al.*, 1986).

The functional properties of most of the purified glycosomal enzymes have been analyzed in detail (Misset and Opperdoes, 1987; Misset *et al.*, 1987; Marchand *et al.*, 1989; Lambeir *et al.*, 1987, 1991; Cronin and Tipton, 1985; 1987). These analyses have shown that the kinetic parameters of some of these enzymes (PGI and TIM) are rather similar to those of their mammalian counterparts, whereas other enzymes (PFK, ALD, gGADPH, and gPGK) display more unusual characteristics. This dissimilar kinetic behavior should be attributed to the participation of some different residues in the catalytic process. For instance, some active-site residues which directly participate in the catalytic reaction could be different in the glycosomal enzymes; or different amino acids are involved in cofactor binding or channeling of substrates to the active site. Several glycosomal enzymes have been crystallized and the three-dimensional structure of two (TIM, GAPDH) has been solved (Wierenga *et al.*, 1987; 1991; Vellieux *et al.*, 1993).

BIOSYNTHESIS OF *T. BRUCEI* GLYCOSOMAL ENZYMES

Glycosomal enzymes are synthesized in the cytosol on free polysomes, and then transferred into the glycosomes. The polypeptides are synthesized at their mature size and do not undergo any detectable proteolytic processing or secondary modification, such as phosphorylation, glycosylation, or covalent linkage of lipids upon transfer into the glycosomes. It must be concluded, therefore, that all information required for import of glycosomal proteins into the organelles resides in the primary structure of the proteins themselves. The half-life of the various proteins in the cytosol is short: between 1.0 and 3.4 min. (Hart *et al.*, 1987; Clayton, 1987). These values are comparable to the fastest translocation rates described for rat-liver or yeast microbody proteins, indicating that the import into the glycosomes is a rapid and efficient process. Conflicting data have been reported about the half-life of glycosomal polypeptides inside the organelle. In pulse-chase and continuous labeling experiments with procyclic trypanosomes, Hart *et al.* (1987) measured half-lives as short as 30 min to 1 h. This suggests that the organelle itself turns over at a rather high rate. Such a high turnover rate is compatible with the calculated rate of synthesis of the glycosomal polypeptides, which would allow the replacement of 1% of the total enzyme pool per minute. In contrast, the half-life of rat-liver peroxisomal proteins has been estimated at 1.5 days. This feature would clearly differentiate glycosomes from the peroxisomes of other eukaryotes. However, the reliability of these data can be questioned because the authors also measured a half-life of 1.5 h for tubulin, which would normally be regarded as a stable component. Moreover, Clayton (1987, 1988) did not detect any significant turnover of glycosomal proteins in bloodstream form and procyclic *T. brucei* during 3-h labeling experiments.

TOPOGENIC SIGNALS IN GLYCOSOMAL ENZYMES OF *T. BRUCEI*. CONSERVATION IN KINETOPLASTIDA

It was recently shown that a common microbody targeting signal, the C-terminal tripeptide -SKL, or some variation thereof, can also operate in *T. brucei* to route an attached reporter protein to the glycosome (Fung and Clayton, 1991; Blattner *et al.*, 1992; Sommer *et al.*, 1992). However, the spectrum of variants that is functional in *T. brucei* differs markedly from that observed for mammalian cells.

The first amino acid can be serine, alanine, or cysteine, as for peroxisomes, or a small neutral amino acid (G, H, N, P, T). Requirements at the second position are very relaxed and the C-terminal leucine can be replaced by other hydrophobic amino acids (isoleucine, tyrosine, or methionine) without much effect on glycosomal targeting. Sequences similar to -SKL have only be detected at the C-terminus of 3 glycosomal proteins: gGAPDH (-AKL), PGI (-SHL) and PGK (-SSL) (Michels *et al.*, 1986; Marchand *et al.*, 1989; Osinga *et al.*, 1985). None of the other glycosomal proteins sequenced in *T. brucei* contains a C-terminal -SKL-like motif. It seems that the trypanosomatids compensate for the relaxed specificity for a -SKL-like signal by having more stringent context requirements. In their transient transfection studies, Blattner *et al.* (1992) have found that some of the -SKL variants, including -SKL itself, do not work as efficiently in trypanosomes as the PGK glycosomal targeting signal. Although -SSL is functional, it is clearly reinforced by the upstream sequence. In this context, it is notable that of the glycosomal proteins so far sequenced, GAPDH shares with PGK the basic amino acid at position -5 relative to the C-terminus. PGI, however, has an acidic residue at this position. This was not tolerated upstream of -SSL but appears not to affect the function of -SHL. It has been shown that the SKL-like motif has been conserved in the glycosomal GAPDH of other Kinetoplastida. The tripeptide is -SKM in *L. mexicana*, -ARL in *T. cruzi*, and -AKL in *T. borelli* (Hannaert *et al.*, 1992; Kendall *et al.*, 1990; E. Wiemer, V. Hannaert and P. Michels, unpublished data).

Evidence has been obtained that -SKL-like signals are not the only microbody targeting signals (Swinkels *et al.*, 1991). Rat ketoacylthiolase contains its signal in a cleavable N-terminus, which shows some similarity to the N-terminus of several other microbody proteins including *T. brucei* aldolase (Clayton, 1985; Marchand *et al.*, 1988). The signal for the PGK-A gene must reside somewhere else than at the C-terminus, perhaps in the central insertion (Alexander and Parsons, 1991). Another probable entry signal is to be found in the 39-amino acid extension at the C-terminus of the glycosomal PGK of *Crithidia fasciculata* (Swinkels *et al.*, 1988): its final 6 amino acids are -MVLASP.

GLYCOSOMES IN OTHER KINETOPLASTIDA

Glycosomes have been detected in all Kinetoplastida examined. In *Leishmania major* promastigotes 50–100 glycosomes were estimated per cell (Hart and Opperdoes, 1984), while serial sections of *L. mexicana* amastigotes revealed only 10 glycosome-like organelles (Tetley and Coombs, 1983). Although this seems a small number compared to that of the promastigote stage, or the various stages of *T. brucei*, it still represents 1% of the total cell volume, because of the relatively small size of this intracellular stage. Subcellular fractionation experiments carried out on promastigote forms of various representatives of the genus *Leishmania* have shown that the glycosomes of the different species resemble each other (Hart and Opperdoes, 1984). They all contain the majority of the enzymes of the glycolytic pathway as well as GDH and glycerol kinase (GK). This suggests that the metabolic pathways associated with the glycosomes of *Leishmania* are similar to those described for the African trypanosome. Glycolytic enzymes have also been detected in intracellular compartments of *L. mexicana* amastigotes (Mottram and Coombs, 1985), but the available information of the biochemical composition of the organelles in this life stage is rather limited.

Taylor *et al.* (1979) have presented evidence for the presence of glycosomes in *C. fasciculata* and in all stages of the life cycle of *T. cruzi*. Although the enzymatic content of the organelles of *C. luciliae* seems similar to that of *T. brucei* glycosomes, no evidence has been found that they contain GK (Opperdoes, 1981); this organism is not capable of anaerobic dismutation of glucose into pyruvate and glycerol.

Electron microscopic analysis has shown that glycosomes are abundantly present in *Phytomonas* sp. isolated from the plant *Euphorbia characias*. The first seven enzymes of the glycolytic pathway, the first two enzymes of glycerol metabolism, PEPCK, MDH, and adenylate kinase are all associated with these glycosomes (Sanchez-Moreno *et al.*, 1992). The energy metabolism of this plant parasite resembles that of the bloodstream form of *T. brucei* not only by the localization of the enzymes, but also by its very high glycolytic capacity.

Glycolytic enzymes were also found to be associated with the organelles in the fish parasite *T. borelli* (Opperdoes *et al.*, 1988). However, the glycolytic activity in this organism was only very low.

DUAL LOCALIZATION OF GLYCOLYTIC ENZYMES IN KINETOPLASTIDA

In all Kinetoplastida studied, GADPH and PGK

activities could be detected in both the glycosomes and the cytosol. For *T. brucei*, *L. mexicana*, and *C. luciliae* it has been shown that these activities should be attributed to different isoenzymes (Misset and Opperdoes, 1987; Misset *et al.*, 1987; Michels *et al.*, 1991; Hannaert *et al.*, 1992; Swinkels *et al.*, 1988). In contrast, Western blot analysis indicated that the GAPDH activity in the cytosol and glycosomes of *T. borelli* is due to identical or, at least, very similar proteins, related to the glycosomal GAPDH of Trypanosomatidae (E. Wiemer, V. Hannaert, and P. Michels, unpublished data). Furthermore only genes related to the glycosomal GAPDH genes of Trypanosomatidae were detected in this organism.

Contrary to the situation described for *T. brucei* bloodstream forms, not only GAPDH and PGK, but also various other glycolytic enzymes, such as TIM and PGI, displayed considerable activity in the soluble fraction of four different *Leishmania* species. These results were obtained by subcellular fractionation of promastigotes using both differential centrifugation, isopycnic centrifugation (Hart and Opperdoes, 1984), and by sequentially disrupting the various membrane systems with digitonin (Kohl *et al.*, 1994; K. Nyame and P. Michels, unpublished data). A similar dual localization has been described in the case of *C. luciliae* (Opperdoes, 1981). Despite the presence of TIM and PGI in the two cell compartments of *L. mexicana*, only one gene could be detected for each of these enzymes (Kohl *et al.*, 1994; K. Nyame and P. Michels, unpublished data). This suggests that only a part of the newly synthesized protein is transferred from its site of synthesis, the cytosol, into the glycosome.

How a single enzyme would distribute over two cell compartments is not yet clear. The glycosomal targeting signal in TIM is not yet known. But both *L. mexicana* PGI and *T. borelli* GAPDH have a C-terminal tripeptide that has been shown to be very effective in targeting reporter proteins to glycosomes of *T. brucei* (Blattner *et al.*, 1992; Sommer *et al.*, 1992). Moreover, the tripeptide of *T. borelli* GADPH is identical to the C-terminal sequence of the *T. brucei* enzyme that is only found in glycosomes. As discussed above, the upstream sequences may be involved in determination of the efficiency. Alternatively, a part of the protein may undergo some minor modification, retaining it in the cytosol.

The possible function of the presence of glycolytic enzymes in two intracellular compartments of various Kinetoplastida is discussed in the accompanying paper (Michels and Hannaert, 1994).

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