Metabolic functions of glycosomes in trypanosomatids

Paul A.M. Michels a,⁎, Frédéric Bringaud b, Murielle Herman a, Véronique Hannaert a

a Research Unit for Tropical Diseases, Christian de Duve Institute of Cellular Pathology and Laboratory of Biochemistry, Université catholique de Louvain, ICP-TROP 74.39, Avenue Hippocrate 74, B-1200 Brussels, Belgium

b Laboratoire de Génomique Fonctionnelle des Trypanosomatidés, Université Victor Segalen Bordeaux 2, UMR-5162 CNRS, 146 rue Léo Saignat, 33076 Bordeaux cedex, France

Received 30 April 2006; received in revised form 17 August 2006; accepted 18 August 2006

Abstract

Protozoan Kinetoplastida, including the pathogenic trypanosomatids of the genera Trypanosoma and Leishmania, compartmentalize several important metabolic systems in their peroxisomes which are designated glycosomes. The enzymatic content of these organelles may vary considerably during the life-cycle of most trypanosomatid parasites which often are transmitted between their mammalian hosts by insects. The glycosomes of the Trypanosoma brucei form living in the mammalian bloodstream display the highest level of specialization; 90% of their protein content is made up of glycolytic enzymes. The compartmentation of glycolysis in these organelles appears essential for the regulation of this process and enables the cells to overcome short periods of anaerobiosis. Glycosomes of all other trypanosomatid forms studied contain an extended glycolytic pathway catalyzing the aerobic fermentation of glucose to succinate. In addition, these organelles contain enzymes for several other processes such as the pentose-phosphate pathway, β-oxidation of fatty acids, purine salvage, and biosynthetic pathways for pyrimidines, ether-lipids and squalenes. The enzymatic content of glycosomes is rapidly changed during differentiation of mammalian bloodstream-form trypanosomes to the forms living in the insect midgut. Autophagy appears to play an important role in trypanosomatid differentiation, and several lines of evidence indicate that it is then also involved in the degradation of old glycosomes, while a population of new organelles containing different enzymes is synthesized. The compartmentation of environment-sensitive parts of the metabolic network within glycosomes would, through this way of organelle renewal, enable the parasites to adapt rapidly and efficiently to the new conditions.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Trypanosome; Glycosome; Glycolysis; Differentiation; Metabolic adaptation; Organelle turnover

1. Introduction

Glycosomes were initially discovered in Trypanosoma brucei [1], the parasite that causes human African sleeping sickness. These organelles appeared to contain most of the glycolytic enzymes, hence their designation as glycosomes. Compartmentation of glycolysis within organelles is unique; in other organisms this metabolic process is essentially cytosolic. Glycosomes were subsequently also found in related protozoa (reviewed in [2–7]), all belonging to the Kinetoplastida, a group that also comprises other parasites causing serious tropical diseases in humans such as Trypanosoma cruzi and a variety of Leishmania species, as well as parasites of other vertebrates, invertebrates and plants, and free-living organisms. These organelles belong to the peroxisome family, although initially some doubt existed about this affiliation because of the absence of catalase, the hallmark peroxisomal enzyme, from Trypanosoma and Leishmania glycosomes and the almost exclusively glycolytic role of the organelles in T. brucei; glycolytic enzymes comprise up to 90% of the glycolosomal protein content when these parasites live in the mammalian bloodstream. Yet, like peroxisomes, these organelles are bounded by a single phospholipid bilayer, contain no DNA and catalase could be detected in glycosomes of some other kinetoplastids. Also

Abbreviations: ATG, autophagy-related gene; Gly3P, glycerol 3-phosphate; DHAP, dihydroxyacetone phosphate; FH, fumarase; FRD, fumarate reductase; GAT, glycosomal ABC transporter; GPO, glycerol-3-phosphate oxidase; HXK, hexokinase; MCF, mitochondrial carrier family; PAT, peroxisomal ABC transporter; PEP, phosphoenolpyruvate; PEPCK, PEP carboxykinase; PGK, phosphoglycerate kinase; PFK, phosphofructokinase; PPDK, pyruvate phosphate dikinase; PTS, peroxisome-targeting signal; PYK, pyruvate kinase; TAO, trypanosome alternative oxidase; TCA, tricarboxylic acid

⁎ Corresponding author. Tel.: +32 2 764 74 73; fax: +32 2 762 68 53.
E-mail address: michels@bchm.ucl.ac.be (P.A.M. Michels).

0167-4889/ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2006.08.019

Please cite this article as: Paul A.M. Michels et al., Metabolic functions of glycosomes in trypanosomatids, Biochimica et Biophysica Acta (2006), doi:10.1016/j.bbamcr.2006.08.019
enzymes of ether-lipid metabolism are often present in glycosomes [8,9], although not in those of bloodstream-form *T. brucei*. Furthermore, subsequent research showed that the biogenesis of glycosomes occurs along similar routes as that of peroxisomes, and involves homologous proteins (peroxins) (reviewed in [7]), and more recent biochemical studies as well as genomic and proteomic analyses revealed more similarities at the metabolic level between glycosomes and other members of the peroxisome family (see below, Sections 5, 6 and 7).

2. Glycosomes during the life-cycle of trypanosomatids

Many Kinetoplastida have a complicated life-cycle [10,11]. This holds particularly true for the best-studied kinetoplastid organisms, the parasites belonging to the genera *Trypanosoma* and *Leishmania*. These organisms are transmitted between their mammalian hosts by insects. Both in the mammal and the insect, they often transit through different parts of the body, each time being exposed to highly different environmental conditions. Most transitions involve the cellular differentiation of the parasite enabling it to adapt to the new environment encountered, or to prepare itself for the next one. For example, *T. brucei* is introduced into the mammalian bloodstream by the bite of a tsetse fly. There it finds a largely constant, glucose-rich environment, allowing it to proliferate rapidly while producing all its ATP by a high rate of aerobic glycolysis or, at places where the oxygen level is low, by anaerobic glycolysis. Sequestering of the major part of the glycolytic pathway inside glycosomes has been shown essential for glycolysis, ATP production and growth of these bloodstream-form trypanosomes [12–14], and provides them with a unique mechanism for quickly switching from aerobic to anaerobic glycolysis (see Section 3) [1,15,16]. This mechanism allows the trypanosomes to overcome short periods of anaerobiosis, although it does not enable them to synthesize sufficient ATP for sustaining growth [17]. Mitochondrial systems such as the tricarboxylic acid (TCA) cycle, respiratory chain and the oxidative phosphorylation machinery are largely repressed at this stage of the life-cycle (reviewed in [3,18–20]). Such glycolysis-dependent, long-slender trypanosomes are not prepared for life in the insect midgut, where the concentration of sugars is usually very low and that of amino acids, notably proline and threonine high; therefore, they quickly die when ingested by a next fly taking a meal. However, in the bloodstream the long-slender forms may differentiate into short-stumpy trypanosomes which are still glycolysis-dependent but have a partially de-repressed mitochondrial system, allowing them to survive in the insect midgut. Upon ingestion, these short-stumpy trypanosomes differentiate rapidly into so-called procyclic forms with a much more elaborate mitochondrial system, and also a considerably different, more extensive glycosomal metabolic network (see Sections 4 and 5). In a period of 2–3 weeks, the trypanosomes move from the midgut, via the foregut and proboscis to the salivary glands, while undergoing additional differentiation steps resulting in morphologically distinct forms [21] which so far have not yet been metabolically characterized. It is likely that the metabolic repertoire of the glycosomes varies with the different environments encountered by each of these differentiated forms. The mammalian-infective metacyclic form, that finally develops in the salivary glands, is prepared again for an exclusively glycolytic metabolism in the mammalian blood.

In contrast to *T. brucei*, which resides extracellularly throughout its life-cycle, *T. cruzi* and *Leishmania* species have additional, intracellular stages in their mammalian hosts: *T. cruzi* in the cytosol of various tissues, *Leishmania* in the lysosomes of macrophages. Consequently, their metabolism has to be able to rely also on the nutrients available in these highly specific environments, with consequences for the adaptability of their metabolism. Moreover, these parasites are transmitted by different insects (triatomine bugs and sand flies, respectively) and follow also different tracks within the insect vector. A need for metabolic adaptability to cope with different environments is possibly also important for each of the many other, biochemically less well-characterized kinetoplastids irrespective whether they infect one or more specific hosts or are free living. As will be argued below, glycosomes are likely to play an important role in the metabolic adaptation to each of the environments to which kinetoplastid organisms are exposed.

3. Compartmentation of glycolysis in glycosomes in bloodstream-form *T. brucei*

When parasitizing the mammalian bloodstream, the African trypanosome *T. brucei* is, for its free-energy needs, totally dependent on a continuous supply of glucose present in the blood and body fluids of its host. All ATP synthesized by this organism comes from the conversion of glucose into pyruvate, the end-product of trypanosome glycolysis in this vertebrate stage. The seven enzymes involved in the conversion of glucose into 3-phosphoglycerate are present inside the glycosome, while those catalyzing the last part of the pathway are localized in the cytosol (Fig. 1) [1]. The pyruvate is excreted into the host’s bloodstream. Within the glycosome, consumption and production of ATP and NADH by glycolysis are balanced: the intraglycosomal consumption of ATP by hexokinase (HXK) and phosphofructokinase (PFK) is compensated by the ATP production by phosphoglycerate kinase (PGK). Net ATP formation occurs in the cytosol, in the reaction catalyzed by pyruvate kinase (PYK). Similarly, the glycosomal NADH resulting from the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase is re-oxidized inside the organelle followed by the transfer of the electrons to O2 via a mitochondrial glycerol-3-phosphate oxidase (GPO). This process involves a glycosomal NADH-dependent glycerol-3-phosphate dehydrogenase, a putative transporter (‘shuttle’) in the glycososomal membrane which exchanges glycerol 3-phosphate (Gly3P) for dihydroxyacetone phosphate (DHAP), and the mitochondrial GPO. The GPO is in fact a system comprising an FAD-linked glycerol-3-phosphate dehydrogenase that is most likely associated with the outer surface of the mitochondrial inner membrane, ubiquinone and a terminal oxidase, known as the trypanosome alternative oxidase (TAO). This respiratory process seems not to be involved in free-energy transduction. The TAO is insensitive to cyanide, but can be
inhibited by salicylhydroxamic acid. Inhibition of TAO mimics the effect of a lack of oxygen on carbohydrate metabolism of the bloodstream form. Under these conditions, NAD⁺ is regenerated by the reduction of DHAP to Gly3P, followed by the formation of glycerol that is excreted by the organism. This is achieved by reversal of the glycerol kinase reaction, thus synthesizing glycerol and ATP from Gly3P and ADP [1,15,16]. However, this reverse reaction is thermodynamically unfavourable and only feasible by mass action at high Gly3P concentration and a high ADP/ATP ratio, conditions that are difficult to attain in an entire cell but may occur in a small, closed compartment. By this reverse reaction, the ATP/ADP balance within the glycosomes is maintained; the ATP formed in the glycerol kinase reaction compensates for the loss of one molecule of ATP in the PGK reaction, because now one molecule of 3-phosphoglycerate, rather than two, is produced per molecule of glucose consumed. As a consequence, glucose is dismutated into equimolar amounts of pyruvate and glycerol, with net synthesis of only one molecule of ATP.

The regulation of glycolysis in trypanosomes differs largely from that of other organisms. There is an apparent lack of activity regulation of the glycolytic enzymes HXK and PFK [22–24]. In most organisms, the activities of these two key enzymes are highly regulated by their products, by metabolites further downstream in the metabolism or by other (allosteric) effectors [25]. This regulation serves two purposes. First, it prevents the loss of ATP by futile cycling when glycolysis and gluconeogenesis occur simultaneously. Second, it has been
argued, and experimentally confirmed by using Saccharomyces cerevisiae mutants, that the ‘turbo design’ of glycolysis requires a tight regulation of the first steps of glycolysis [26]. Indeed, absence of regulation may lead to unrestricted accumulation of glycolytic intermediates, a situation that will be highly toxic for the cell.

Proper compartmentation of glycolytic enzymes inside glycosomes and possession of intact organelles are essential for the survival of bloodstream-form T. brucei. Indeed, several experiments have provided evidence that mislocalization of glycolytic enzymes is deleterious for the parasite. Trypanosomes contain two major isoenzymes of PGK located in different compartments of the cell: one (PGKg), present in glycosomes, is only expressed in the mammalian bloodstream-form parasites, while a cytosolic enzyme (PGKc) operates predominantly in procyclic trypanosomes [27,28]. Bloodstream-form trypanosomes were rapidly killed when, from an additional ectopic gene, PGKc or a truncated form of PGKg without the glycosome-targeting signal (PTS1) was expressed [29]. Expression of an inactive PGKc had no effect, indicating that the toxicity depends both on enzyme activity and cytosolic location. Similar growth inhibition has been observed after transfection of bloodstream-form T. brucei with the gene of S. cerevisiae triosephosphate isomerase expressed as a cytosolic enzyme [17]. Furthermore, partial relocation of glycosomal matrix proteins to the cytosol upon depletion of peroxins PEX2, 6, 10, 12 or 14 by RNA interference (RNAi) readily killed bloodstream-form cells [13,14,30,31].

To understand why the presence of a glycolytic enzyme in the wrong compartment is detrimental for trypanosomes, one has to know the function or consequences of this form of metabolic compartmentation. To this aim, a mathematical model of glycolysis in bloodstream-form T. brucei was developed on the basis of kinetic data as determined for the different glycolytic enzymes [32]. One question addressed was how the functional behaviour of trypanosome glycolysis would change if the pathway were not compartmentalized [12]. According to the computer model this would not significantly affect the steady-state glycolytic flux. But, strikingly, it will lead to toxic accumulation of hexose-phosphates upon addition of glucose. Such toxic accumulation is prevented by compartmentation, since the kinases at the beginning of the pathway respond to the glycosomal ATP/ADP ratio, not the cytosolic one. The glycosomal ATP/ADP ratio, which, according to the computer model, is usually low, controls the activity of HXK and PFK and maintains the concentration of the hexose-phosphates constrained within a narrow range. If the enzymes would sense the higher cytosolic ATP/ADP ratio, their activity would not be restrained and the intermediates would accumulate. Therefore, the computer analysis suggested that the sequestering of the glycolytic pathway within a membrane that is poorly permeable for solutes is essential for the parasite, because it compensates for the lack of activity regulation of its enzymes. Indeed, experimental data have been obtained to support the notion that compartmentation of glycolysis serves such a regulatory function and will be presented in Section 4.

4. Glycosomes and energy metabolism in insect-stage T. brucei and other trypanosomatids

During its journey through the tsetse fly, T. brucei undergoes several differentiation steps, adapting itself to each new environment encountered. The procyclic form living in the midgut is, with the bloodstream form, the only one that so far could be axenically cultured and has thus been made amenable to biochemical investigations. Also the promastigote Leishmania spp. and epimastigote T. cruzi, which propagate in the midgut of their respective insect vectors, are commonly studied in laboratories. Although the metabolic networks, both within the glycosomes and the fully developed mitochondrion, of all these insect-stage trypanosomatids seem very similar, most of the recent data regarding glycosomes and energy metabolism pertain to procyclic trypanosomes, since gene silencing by RNAi could be extensively used in T. brucei [33,34], but failed to be functional in the other parasites [35,36].

When grown in commonly used glucose-rich media, procyclic T. brucei and epimastigote T. cruzi primarily use the glucose as carbon source [37,38], whereas amino acids (including L-proline) are the main energy source present in their insect vector [39]. In glucose-depleted medium, however, procyclic trypanosomes considerably increase the rate of L-proline consumption (6-fold), indicating that it is the major carbon source used by the parasite in its natural environment and glucose, when present, exerts a negative control on L-proline metabolism [40]. Glucose is catabolized in the glycosomes, as described above for the bloodstream-form trypanosome (see Section 3), whereas L-proline degradation takes place in the mitochondrion. Glycolysis appeared dispensable, since procyclics of several strains have been successfully adapted to glucose-depleted media with no significant effect on growth rate [40,41]. Interesting is the non-essential role of glycosomes in procyclic trypanosomes grown in glucose-depleted media, as exemplified by the analysis of a PEX14 knock-down mutant [14]. Procyclic trypanosomes were killed by RNAi-dependent depletion of PEX14 only when glucose was present, suggesting that glycosomes are only essential to compartmentalize a functional glycolytic pathway. This observation strengthens the hypothesis that glycosomes serve to prevent the dangerous effect of the turbo design of glycolysis [12,26] (see Section 3). Experimental support for this hypothesis was provided by the observation that RNAi-mediated knockdown of HXK rescued procyclic cells from glucose toxicity, even though glycosomal proteins were mislocalized to the cytosol [42].

The extensive trachal system of insects renders all organs and tissues, including the digestive tractus, highly aerobic [43]. Consequently, insect parasites have developed an aerobic metabolism, exemplified by the presence of two distinct mitochondrial terminal oxidases (the cyanide-sensitive cytochrome oxidase and the salicylhydroxamic acid-sensitive TAO), both using oxygen as electron sink. Indeed, the midgut trypanosomatids cannot adapt to long-term growth under anaerobic conditions and go into reversible or irreversible metabolic arrest during anoxia [44–46]. Although they are dependent on oxygen
for proliferation, the insect-stage trypanosomatids developed a fermentative-like metabolism, since glucose (and L-proline) is converted into partially oxidized end-products (succinate, acetate, lactate, ethanol and/or glycerol, depending on the species) by so-called aerobic fermentation [47].

In these insect-stage trypanosomes too, glucose is essentially metabolized inside the glycosomes (Fig. 2), but with three main differences compared with the bloodstream form of the parasite (for recent reviews see [5,18–20]). First, PGK is present in the cytosol and, therefore, 3-phosphoglycerate is produced in this compartment [27]. Second, phosphoenolpyruvate (PEP) produced in the cytosol forms a metabolic branching point and is the substrate for two glycosomal kinases, i.e., PEP carboxykinase (PEPCK) and pyruvate phosphate dikinase (PPDK) [48–50]. The PEP-CO₂ condensation catalyzed by PEPCK is the initial step to the production, inside glycosomes, of succinate, a major end-product excreted by most trypanosomatids [20,46,47,51,52]. Third, instead of being the final end-product, pyruvate is further converted into acetate, lactate and L-alanine in the mitochondrion and/or the cytosol [46,51,53,54]. The two former changes imply that the mechanism by with the redox potential and ATP/ADP ratio are balanced within glycosomes of procyclic trypanosomes differs from that in the bloodstream form of *T. brucei*.

The production of succinate from PEP involves two glycosomal NADH-dependent oxido-reductases, malate dehydrogenase and fumarate reductase (FRDg) [49,51], which provide a means for the re-oxidation of NADH produced in

---

**Fig. 2.** Schematic representation of glucose metabolism in the procyclic form of *T. brucei*. Excreted end products of glucose metabolism (acetate, L-alanine, glycerol, L-lactate, succinate and CO₂) are in white characters on a black background. Arrows with different thicknesses tentatively represent the metabolic flux at each enzymatic step. Dashed arrows indicate steps which are supposed to occur at a background level or not at all. The mitochondrial outer membrane is permeable to metabolites and is only shown in the vicinity of the schematic electron-transport chain. Abbreviations: AA, amino acid; 1,3BPGA, 1,3-bisphosphoglycerate; C, cytochrome c; CoASH, coenzyme A; DHAP, dihydroxyacetone phosphate; F-6-P, fructose 6-phosphate; FBP, fructose 1,6-bisphosphate; G-3-P, glyceraldehyde 3-phosphate; G-6-P, glucose 6-phosphate; Gly-3-P, glycerol 3-phosphate; OA, 2-oxoacid; PEP, phosphoenolpyruvate; 3-PGA, 3-phosphoglycerate; Pi, inorganic phosphate; PPi, inorganic pyrophosphate; SucCoA, succinyl-CoA; UQ, ubiquinone pool. Enzymes are: 1, hexokinase; 2, glucose-6-phosphate isomerase; 3, phosphofructokinase; 4, aldolase; 5, triosephosphate isomerase; 6, glycol-3-phosphate dehydrogenase; 7, glyceraldehyde 3-phosphate dehydrogenase; 8, glycosomal phosphoglycerate kinase; 9, cytosolic phosphoglycerate kinase; 10, phosphoglycerate mutase; 11, enolase; 12, pyruvate kinase; 13, phosphoenolpyruvate carboxykinase; 14, pyruvate phosphate dikinase; 15, pyruvate phosphate dikinase; 16, glycosomal malate dehydrogenase; 17, cytosolic and glycosomal fumarase (FHc); 18, glycosomal NADH-dependent fumarate reductase; 19, mitochondrial fumarase (FHm); 20, mitochondrial NADH-dependent fumarate reductase; 21, glyceraldehyde kinase; 22, malic enzyme; 23, unknown enzyme; 24, alanine aminotransferase; 25, pyruvate dehydrogenase complex; 26, acetate:succinate CoA-transferase; 27, unknown enzyme; 28, succinyl-CoA synthetase; 29, FAD-dependent glycerol-3-phosphate dehydrogenase; 30, rotenene-insensitive NADH dehydrogenase; 31, alternative oxidase; 32, Fd,F₄-ATP synthase; I, II, III and IV, complexes of the respiratory chain.
the organelle by the glycolytic glyceraldehyde-3-phosphate dehydrogenase. Theoretically, two NADH molecules are consumed per molecule of succinate produced for a single NADH molecule produced per molecule of PEP synthesized from glucose, implying that only half of PEP produced in the cytosol needs to be re-routed to the organelle and converted into succinate in order to balance the NADH/NAD⁺ ratio. However, the fraction of PEP metabolized in the glycosome is significantly higher than expected, since approximately 70% of the glucose consumed is converted into succinate [51,55]. This apparent discrepancy probably results from the existence of a second succinate production pathway, located in the mitochondrion, fed by malate produced in the glycosomes (Fig. 2), which accounts for 14–44% of succinate produced from the glucose metabolism [52]. For each succinate molecule produced in the mitochondrion, a single NADH molecule is formed in the glycosomes, instead of two. Assuming that the glycosomal succinic fermentation is sufficient for maintaining the glycosomal ATP/ADP balance, as confirmed experimentally, the PPDK activity is probably not sufficient to maintain the ATP balance inside the glycosomes of procyclic trypanosomes [4,5,46,47]. PPDK is another glycosomal ATP-producing enzyme, which utilizes PEP, AMP and Pi to produce pyruvate, ATP and Pi [50,63]. This PPI-dependent enzyme was proposed to substitute for pyrophosphatase in order to hydrolyze the PPI produced in the glycosomes by biosynthetic pathways (see Section 5) which would otherwise be inhibited [4,5,50,63]. This hypothesis implies that the rate of ATP production by PPDK depends on the flux of the PPI-generating pathways, which should be significantly lower than the catabolic flux of glucose. Consequently, the PPDK activity is probably not sufficient to maintain the glycosomal ATP/ADP balance, as confirmed experimentally, since the viability and glucose metabolism are not affected by PPI gene knockout [38]. Alternatively, ATP could be imported from outside the glycosomes, similar as reported for peroxisomes, where the yeast Ant1p and human PMP34 nucleotide transporters are involved in the exchange of AMP-ADP and ATP across the membrane, to provide intraperoxisomal ATP for β-oxidation or activation of fatty acids, respectively [64,65]. Indeed, the recent proteomic analysis of glycosomes from procyclic trypanosomes identified a protein homologous to mitochondrial ATP/ADP carriers, but a mitochondrial contamination of the glycosomal-enriched fraction cannot be excluded [58]. The presence of several ATP-producing pathways in addition to a putative nucleotide transporter may provide an important metabolic flexibility for adaptation to rapid environmental changes. However, questions remain as to how the glycosomal ATP/ADP balance is maintained in procyclic trypanosomes.

The relocation of PGK to the cytosol of trypansomes when they differentiate from bloodstream to procyclic forms results from the expression of a different isoenzyme, PGKe instead of PGKg. This change has been interpreted in relation to the necessity to balance the ATP production and consumption inside the glycosomes: in procyclies the phosphorylation of the glycolytically produced ADP would be performed by PEPCK during the glycosomal succinic fermentation. However, the conversion of a significant part of PEP into pyruvate by the cytosolic PYK implies that the PEPCK reaction would not contribute sufficiently to maintaining the glycosomal ATP/ADP balance. Consequently, at least another glycosomal ATP-producing step is required to complete the metabolic scheme. This function may be fulfilled by a constitutively expressed third PGK isoform, the glycosomal PGKA [27,28,61,62]. The dual localization of PGK activity (PGKA and PGKe) may help to maintain the ATP balance inside the glycosomes of procyclic trypanosomes by altering the ratio of the fluxes from 1,3-bisphosphoglycerate to 3-phosphoglycerate via the two different routes (Fig. 2). However, the contribution by PGKA to glycosomal ATP production is debated, since its relative amount is very low compared to the other isoforms in T. brucei, it has a low specific activity, and its activity is not detectable in glycosomal fractions of procyclic trypanosomes [(48), and F.B., unpublished data]. The alternative of glycosomal ATP production by glycerol kinase is thermodynamically unfavoured, and thus unlikely, and indeed the procyelic form does not excrete significant amounts of glycerol when grown under standard conditions [46,47,51]. PPDK is another glycosomal ATP-producing enzyme, which utilizes PEP, AMP and Pi to produce pyruvate, ATP and Pi [50,63]. This PPI-dependent enzyme was proposed to substitute for pyrophosphatase in order to hydrolyze the PPI produced in the glycosomes by biosynthetic pathways (see Section 5) which would otherwise be inhibited [4,5,50,63]. This hypothesis implies that the rate of ATP production by PPDK depends on the flux of the glycosomal PPI-generating pathways, which should be significantly lower than the catabolic flux of glucose. Consequently, the PPDK activity is probably not sufficient to maintain the glycosomal ATP/ADP balance, as confirmed experimentally, since the viability and glucose metabolism are not affected by PPDK gene knockout [38]. Alternatively, ATP could be imported from outside the glycosomes, similar as reported for peroxisomes, where the yeast Ant1p and human PMP34 nucleotide transporters are involved in the exchange of AMP-ADP and ATP across the membrane, to provide intraperoxisomal ATP for β-oxidation or activation of fatty acids, respectively [64,65]. Indeed, the recent proteomic analysis of glycosomes from procyclic trypanosomes identified a protein homologous to mitochondrial ATP/ADP carriers, but a mitochondrial contamination of the glycosomal-enriched fraction cannot be excluded [58]. The presence of several ATP-producing pathways in addition to a putative nucleotide transporter may provide an important metabolic flexibility for adaptation to rapid environmental changes. However, questions remain as to how the glycosomal ATP/ADP balance is maintained in procyclic trypanosomes.
Adaptive forms of all trypanosomatids analyzed to date, except the slender bloodstream form of *T. brucei*, express the NADH-dependent FRD activity [51,66–69] and produce significant amounts of excreted succinate from glucose [20,47]. Interestingly, succinate production seems to be correlated with the presence of a PGK activity in the cytosol, since the bloodstream form of *T. brucei* is the only one lacking the PGKc isoform. All other trypanosomatid forms analyzed express at least one cytosolic PGK isoform, often simultaneously with glycosomal isoforms [27,28,70–72]. In other words, the exclusively glycosomal localization of PGK is restricted to bloodstream-form *T. brucei* and correlates with the absence of the glycosomal succinic fermentation pathway. Thus, the repression of the succinic fermentation pathway by bloodstream-form *T. brucei* is accompanied with the repression of the cytosolic PGK in favour of the glycosomal isoform in order to balance the glycosomal ATP/ADP ratio. These adaptations to the glucose-rich environment of the vertebrate blood came along with the repression of the mitochondrial TCA cycle enzymes and respiratory chain. Interestingly, this expression strategy appears to be a recent evolutionary adaptation by *T. brucei*, because it is not found in all other trypanosomatids including its closest known relative, *T. congolense*, which has a very similar life cycle; it also propagates in the blood of vertebrates after transmission by tsetse flies. The bloodstream form of *T. congolense* excretes succinate and expresses both the glycosomal and cytosolic PGK isoforms [47,70]. The *T. congolense* orthologous gene (c2PGK) of the *T. brucei* glycosomal PGKg gene encodes a cytosolic isoform. Consequently, PGKA is the only glycosomal isoform expressed in *T. congolense* [70]. In conclusion, all trypanosomatids analyzed so far developed the largely glycosomally-located metabolic network for aerobic fermentation of glucose. Only the long-slender bloodstream form of *T. brucei* could afford to considerably reduce this network to the mere glycolytic pathway, possibly as a result of its constant and glucose-rich environment.

5. Other metabolic systems in glycosomes

Besides the glycolytic pathway and its extra branches towards glyceral and succinate production, many other enzymes, often parts of pathways, have been found inside glycosomes during the last 20 years (Fig. 3). Most of these enzymes were not, or only at very low activity, detectable in bloodstream-form *T. brucei*, but were shown to be present in glycosomes of cultured procyclic *T. brucei* and/or insect- or mammalian-stage cells of *Leishmania* species or *T. cruzi*, mostly upon cell fractionation, sometimes by immunofluorescence studies with intact cells. And indeed most of the enzymes were shown to possess a PTS1 or PTS2. For example, the key enzyme of the gluconeogenic pathway, fructose-1,6-bisphosphatase, possesses peroxisome-targeting signals [73] and indeed was demonstrated to be present in glycosomes of *T. brucei*. The observation that this enzyme is expressed simultaneously with the glycolytic PFK (VH, unpublished result), and the apparent absence from trypanosomatid PFK of activity regulation mechanisms found in other organisms [23,24] raise the still unsolved question as to how futile ATP hydrolysis resulting from the combined activity of these two glycosomal enzymes is avoided.

Also another glucose consuming process, the oxidative branch of the pentose-phosphate pathway, is compartmentalized inside glycosomes [74,75]. Although most of the enzymes of this pathway in the three trypanosomatids for which the genome sequencing has been completed appear to have a PTS, often a substantial part of their activity was found in the cytosol, indicating only partial compartmentation; in *T. cruzi* the cytosolic contribution is even predominant [76]. Intriguingly, genes encoding a sedoheptulose-1,7-bisphosphatase with a PTS1 have been found in *T. brucei* and *T. cruzi*. This is a Calvin cycle enzyme, usually only found in plastids of plants and algae and in some fungi and ciliates where it is possibly present in the cytosol exerting a still unknown function. Possibly the trypanosomatids acquired the sedoheptulose-1,7-bisphosphatase by lateral gene transfer [73,77]; we hypothesize that the parasites employ it in a modified pentose-phosphate pathway (Fig. 3A), as described in more detail elsewhere [5].

All trypanosomatids studied contain various isoforms of adenylate kinase, an enzyme that is important for ATP homeostasis. In *T. brucei* seven genes encoding the enzyme for several cell compartments have been found, including one for a glycosomal enzyme [78]. The importance of this enzyme for glycosomal metabolism has been shown by computer simulation of metabolism and expression knockdown using RNAi [32,78].

A glycosomal localization has previously also been reported for most enzymes of the purine salvage pathway in different trypanosomatids and for a bifunctional enzyme, resulting from a gene fusion, catalyzing the last two of the six steps usually comprising the *de novo* pyrimidine biosynthetic pathway, whereas the other four enzymes were found in the cytosol (Fig. 3B and C) (reviewed in [4,79]). Interestingly, the genes of the enzymes for pyrimidine biosynthesis are organized in a highly unusual way for trypanosomatids, i.e., in an operon-like manner, similar to that in cyanobacteria and not found elsewhere [80]. This organization and the sequence similarity strongly suggest that these genes were acquired by lateral gene transfer. As mammalian peroxisomes, trypanosomatid glycosomes have been shown to contain the first two steps of ether-lipid biosynthesis, alkyl-DHAP synthase and DHAP acyltransferase (Fig. 3F) [8,9]. Also common with peroxisomes is the association with glycosomes of some enzymes of β-oxidation of fatty acids, such as 2-enoyl-CoA hydratase and an NADPH-dependent 3-hydroxyacyl-CoA dehydrogenase (Fig. 3E) [81,82]. For *T. brucei* it was shown that these two enzymes behave as a bifunctional enzyme [82].

As mentioned above, the peroxisomal marker enzyme catalase is absent from *Trypanosoma* and *Leishmania* species, but it has been found in other kinetoplastids such as *Crithidia luciliae*, *Phytomonas* sp. and *Trypanosoma borelli*, where it was located in the glycosomes [2,3]. But in recent years, a different peroxide detoxification system has been unravelled in trypanosomatids and components of it were found also in...
the glycosomes, in line with the possession of a PTS by some of them [83]. The unique trypanosomatid system is a cascade comprising a modified form of glutathione, $N_7,N_8$-bis (glutathionyl)spermidine designated trypanothione, trypanothione reductase, a thioredoxin-related protein called tryparedoxin and 2-Cys-peroxiredoxin-type tryparedoxin peroxidases and glutathione peroxidases (Fig. 3H) (reviewed in [84]). Other enzymes involved in oxidative stress defence have been detected in glycosomes such as one of the four iron-dependent superoxide dismutase isoenzymes of trypanosomatids [85,86].

The recently completed genome sequencing projects for T. brucei, T. cruzi and L. major [87] have allowed to get further insight into the metabolic repertoire of glycosomes by searching all protein sequences with either a potential C-terminal PTS1 or an N-terminal PTS2 [80]. Moreover, proteomic analyses of glycosomes purified from both bloodstream- and procyclic-form T. brucei have been initiated [58]. In the bioinformatic analysis of L. major, 191 potential PTS1-containing proteins and 68 potential PTS2-containing proteins with homologues in T. brucei and T. cruzi were identified. About 50% of these were hypothetical proteins to which no function could be attributed as...
yet. The results of these genomic and proteomic studies largely confirm the earlier experimental findings described above, while filling in some gaps in pathways (Fig. 3). However, the proteomic study did not detect any enzymes of β-oxidation, although two enzymes were found with a candidate PTS2 in all three trypanosomatids: the bifunctional 2-enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase mentioned above and 3-ketoacyl-CoA thiolase. Possibly, the proteomic analysis was performed with trypanosomes cultured under conditions by which the pathway was repressed. However, the bifunctional enzyme may also be targeted differently, since its sequence contains also a candidate mitochondrial transit peptide. Additionally, the genome sequences of *T. cruzi* and *L. major* revealed candidate glycosomal enzymes that should allow these parasites to feed the glycolytic pathway also with various other sugars than glucose [80].

The glycosome also harbours some enzymes of the mevalonate pathway for the biosynthesis of isoprenoids, precursors of many important compounds such as squalene and eventually sterols (Fig. 3G). Three enzymes, responsible for the conversion of mevalonate to 5-pyrophospho-mevalonate were each shown to possess a PTS in at least two of the three trypanosomatids. The same holds true for a fourth enzyme further in the pathway, squalene synthase, converting farnesy1-pyrophosphate into squalene. The first enzyme of the pathway, 3-hydroxy-3-methyl-glutaryl-CoA reductase had previously been localized to mitochondrion and glycosomes [88]; its sequence has indeed a candidate mitochondrial transit peptide but not an identifiable PTS. The proteomic analysis detected the mevalonate kinase only in glycosomes of procyclic cells.

In addition to adenylate kinase mentioned above, glycosomes may contain another enzyme involved in ATP homeostasis, arginine kinase (Fig. 3H). *T. brucei* and *T. cruzi*, but not *Leishmania* species, contain this enzyme; in *T. brucei* even three genes were identified coding for enzymes differing only in their N- and C-termini. One of them has a putative PTS1.
Indeed, the proteomic analysis identified the enzyme in glycosomes of bloodstream- but not procyclic-form trypanosomes. Studies with *T. cruzi* have shown that phospho-arginine is particularly important during periods of stress; it is likely that this phosphagen acts as an energy reserve during differentiation steps [89,90].

6. Metabolite transporters in the glycosomal membrane

As for peroxisomes, several lines of evidence provide strong support for the notion that the membrane of glycosomes is poorly permeable (reviewed in [7]). First, the biphasic kinetics of radioactive labelling of glycolytic intermediates, when trypanosomes received a pulse of 14C-glucose, suggested the existence of two pools of intermediates: a rapidly labelled glycosomal one, representing 20–30% of the total pool of glycolytic metabolites and a slowly labelled cytosolic pool representing 70–80% of the intermediates [91]. Exchange between both pools of intermediates appeared to happen only very slowly. Second, under anaerobic conditions bloodstream-form trypanosomes are still capable of performing glycolysis by producing one molecule of pyruvate and one molecule of glycerol per molecule of glucose consumed instead of two molecules of pyruvate. As discussed in Section 3, this involves the reversal of the glycerol kinase reaction, driven by accumulation of Gly3P and a low ATP/ADP ratio as can only be achieved in a small, closed compartment separate from the cytosol. Further strong indications for the glycosomal membrane as a permeability barrier are the above mentioned importance of maintaining a redox (NADH/NAD⁺) balance, the apparent requirement of stoichiometry for the sum of reactions inside the organelle [32], and the observed detrimental effects resulting from mislocalization of glycolytic enzymes [17,29].

The existence of the permeability barrier implies the need for transporter molecules or pores for the exchange of specific substrates, products and intermediates. So far, two groups of candidate solute carriers, homologous to known transporters of peroxisomal membranes [64,92] (see also elsewhere in this issue) have been identified in glycosomes: ABC transporters [93] and MCF proteins belonging to the Mitochondrial Carrier Family [58]. Three so-called half-ABC transporters (called GAT1-3, for Glycosomal ABC Transporter) have been identified in the glycosomal membrane of *T. brucei*, and homologues were found in the genomes of *T. cruzi* and *L. major*. *T. brucei* GAT2 has been shown to complement, to a limited extent, growth on oleate by *Saccharomyces cerevisiae* cells deficient in both peroxisomal fatty-acid transporters PAT1 and PAT2 [94]. GAT1 and GAT3 were not able to complement the deficiency; no information is as yet available about their substrate specificity. GAT1 is predominantly expressed in procyclic *T. brucei*. Interestingly, GAT2 is only expressed in bloodstream-form *T. brucei* in which glycolysis is by large the major function of glycosomes. However, for theoretical reasons, we consider it doubtful that ABC transporters are involved in the transport of glycolytic intermediates. The ATP yield of glycolysis is low: maximally two molecules of ATP per molecule of glucose consumed. Any further decrease of this yield by spending ATP for transmembrane transport processes within the glycolytic process itself would be highly disadvantageous for the cell, and thus unlikely. We therefore consider it more likely that other kinds of transport processes are responsible for the glycolytic intermediates. A first option is the involvement of MCF transporters. Indeed, trypanosomatid genomes contain several MCF homologues, most of them probably present in the inner mitochondrial membrane [87]. But the recent proteomic analysis detected three of them in glycosome preparations of procyclic *T. brucei* [58]. However, none has been found so far in glycosome preparations from bloodstream-form cells. The three MCF molecules found in procyclic glycosomes present the highest percentage of identity/similarity to phosphate carriers, dicarboxylate carriers and ATP/ADP exchangers. However their functional identity remains to be determined and their glycosomal localization confirmed.

Several other possibilities for solute translocation through the glycosomal membrane may be considered. (1) Entry into or exit from glycosomes by diffusion along the (electro)chemical gradient via specific transporters or pores not belonging to a known class of proteins and that remain to be identified (2) Exchange or antiport processes not involving MCF proteins, in which the import of one metabolite is obligatory coupled to the export of another one. Also in this case, the energy would be provided by the transmembrane gradients of the compounds. Kinetic and stoichiometric analyses suggest that such exchange is very likely to happen for the transport of Gly3P and DHAP [95,96]. Antiport systems for other metabolites could be imagined as well. (3) A more speculative possibility is an association of substrate-specific soluble enzymes (in the glycosomal matrix or cytosol) with non-specific carriers or pores in the glycosomal membrane, thus creating a kind of specific ‘group-translocation process’ by which the vectorial process is driven by its coupling to the enzymatic conversion. This might, for example, be the case for glucose uptake and its subsequent phosphorylation by HXK, resembling the PEP-dependent phosphotransferase system responsible for glucose transport in many bacteria [97]. However the glycosomal system would be fundamentally different from the bacterial one, because in trypanosomes glucose phosphorylation is performed by an authentic ATP-dependent HXK. Interestingly, trypanosomatid HXK seems to have affinity for membranes. Furthermore, it could be imagined that also other enzymes may associate with such a non-specific pore or transporter, either other sugar-phosphorylating enzymes or enzymes catalyzing entirely different reactions at the beginning or end of a glycosomal pathway. The former option may be the case in glycosomes of the fish parasite *Trypanoplasma borreli*, where the full set of glycolytic enzymes could be found except HXK [98]. The fact that the metabolic schemes presented above invoke many solute translocation steps through the glycosomal membrane, together with the observed low protein content of the membranes and the difficulty in identifying such candidate transporters by classical biochemical means and modern genomic and proteomic analyses seem to make it worth to experimentally pursue the latter option.
7. Role of glycosomes in metabolic programming during differentiation of trypanosomatids

As described above, the life-cycle of trypanosomatids is highly complex; the parasites are at every step morphologically and metabolically well adapted to a distinct compartment of their specific hosts. As the successive differentiation stages may encounter highly different environments, the parasites have to rapidly change their metabolism to cope with the altered conditions. The compartmentation of environment-sensitive parts of metabolism within glycosomes could be a way by which the trypanosomes achieve such flexibility. As explained in previous sections, the enzymatic content of the organelles may vary considerably from one adaptive form to the other; therefore, it could be imagined that a rapid way to adapt the cell’s metabolism is changing its glycosome population by specifically destroying the old, not anymore adapted organelles and inducing the synthesis of new ones, similar as has been reported for yeast peroxisomes when growth conditions change (Fig. 4).

The peroxisome renewal process has been studied in detail in methylotrophic yeasts. Glucose-grown *Hansenula polymorpha* contains only one or two small peroxisomes. When these cells are transferred to methanol-containing medium, proteins of methanol assimilation are imported so the organelles grow and new organelles are formed by fission from the mature ones [99,100]. After fission the mature organelles lose the capacity to incorporate new protein; protein import is confined to the smaller organelles that have budded off. The other part of the process is the removal of the redundant organelles. The degradation of peroxisomes occurs by a process called pexophagy, a specific form of autophagy, as described in detail elsewhere in this issue (reviewed in [101,102]). Autophagy and pexophagy share several proteins with the cytoplasm-to-vacuole targeting (Cvt) pathway by which some newly synthesized vacuolar proteins of yeasts are routed to their organelles. This specific degradation of peroxisomes can occur by two different ways. The first one, known as micropexophagy, involves the complete engulfment of the organelles by extended vacuole arms. The second one, called macropexophagy is even more selective: the organelles to be degraded are surrounded by membranous layers to form an autophagosome, whose membrane will fuse with that of the vacuole. The induction of either micro- or macropexophagy depends on the nature of a nutrient signal and differs between species. By the combination of selective and induced degradation and synthesis of peroxisomes, yeasts can create a population of organelles with a different set of enzymes, and thus adapt efficiently and rapidly to new environmental conditions.

The situation might be similar for trypanosomatids: the glycosomes may be heterogeneous; a subset of proliferation-competent organelles may give rise to a population with enzymes that allows differentiating parasites to deal with the new conditions encountered by the new life-cycle stage, whereas the old organelles could be degraded specifically. Indeed, recent evidence suggests the presence of autophagy processes in these organisms, particularly during the differentiation from one life-cycle stage into the next one. First, the occurrence of autophagy has been reported previously for *L. major* [103,104], and our recent database searches using the three trypanosomatid genomes against 40 autophagy-related genes (ATG) coding for proteins involved in these processes.

![Fig. 4. Model of the turnover of glycosomes during different steps of the trypanosome’s life cycle. For details, see text. Modified from [108].](image-url)
showed that the autophagy machinery is at least partially present in these organisms [105]. In eukaryotic cells, the Cvt and autophagy pathways can be divided in different steps including the induction and signalling, the cargo selection and packaging, the vesicle nucleation, the retrieval, the docking and fusion, and finally, the vesicle breakdown [106]. Many of the proteins involved in these steps are implicated in different cellular processes, others are specific for one or more forms of autophagy. For each of the different steps, orthologues were either confidently identified in at least one trypanosomatid database or were found to be likely present. Only the Cv pathway seemed absent. However, for each step of autophagy only half of the proteins were identified, suggesting that the autophagy process in trypanosomatids is much simpler than that of yeast, but still viable from a genomic point of view since no key compounds were absent. Recently, autophagy was found to play a role in the differentiation and acquisition of the virulence of *L. major* [107]. An ATG4 null mutant was unable to differentiate into the infective form. Using a fluorescent ATG8 fusion protein as an autophagosome marker, an increase of autophagy was monitored during differentiation. These results confirmed the presence of these two proteins, which were both classified as ‘likely to be present’ in the genomic survey, as well as the presence of a specific autophagy structure, the autophagosomes, in trypanosomatids.

Furthermore, our genomic analysis seemed to indicate that the parasites possess the molecular machinery to degrade selectively their glycosomes by a process analogous to either macro- or microphagophagy. This analysis needs now to be confirmed experimentally. Our (MH and PM) unpublished results with *T. brucei* have revealed that, during the differentiation of this parasite from the bloodstream to the procyclic form, glycosomes co-localize with enlarged lysosomes and glycosomal enzymes specific for the bloodstream form disappear rapidly after differentiation, concomitant with the synthesis of specific procyclic ones. Together, the available data support the hypothesis that glycosomes are rapidly replaced during the transition between different life-cycle stages of the parasites. This process is possibly similar to pexophagy in yeasts; it may be simplified and involve different or additional proteins than the yeast ATG orthologues found in the genomic search. The compartmentation of the enzymes in glycosomes and the rapid replacement of these organelles by others with a different metabolic capacity may therefore augment the adaptability of the trypanosomes to different environmental conditions. Since glycosomes may provide this increased metabolic adaptability to all trypanosomatids and at the various steps when they transform from one life-cycle stage to the next one, it may be considered as a highly important function of these organelles.

8. Conclusions

Glycosomes of Kinetoplastida share some metabolic functions with peroxisomes of other organisms, such as defense against reactive oxygen species and an involvement in ether-lipid biosynthesis and β-oxidation of fatty acids, but they are unique in sequestering the glycolytic pathway, as well as some other parts of the network for carbohydrate metabolism. This subcellular organization of metabolism appears to have some important consequences for these organisms. Proper biogenesis of glycosomes and correct glycosome-targeting of glycolytic enzymes is essential for trypanosomes when grown on glucose, probably because incorrect or incomplete compartmentation affects the regulation of the glycolysis. Moreover, the compartmentation enables these organisms to overcome short periods of anaerobiosis, albeit without sustaining growth. Interestingly, the enzymatic content of glycosomes may vary importantly between different life-cycle stages of parasitic trypanosomatids. Possibly, the sequestering of their pathways for carbohydrate metabolism within glycosomes enables the parasites to adapt rapidly to new nutritional conditions during the differentiation of one life-cycle stage to the next by the ‘en bloc’ degradation of one population of organelles through autophagy, concomitant with the synthesis of a new population of glycosomes with a different enzymatic content. This glycosome-dependent capacity for efficient and rapid metabolic adaptation may have played an important role in the successful radiation of kinetoplastids as parasites with multiple, highly different niches in one or several host species.

Acknowledgements

The research by PM and VH was supported through grants from the ‘Fonds de la Recherche Scientifique Médicale’ (FRSM) of the ‘Communauté française de Belgique’ and the Interuniversity Attraction Poles-Belgian Federal Office for Scientific, Technical and Cultural Affairs. The research by FB received support from the CNRS, the Conseil Régional d’Aquitaine and the Ministère de l’Education Nationale de la Recherche et de la Technologie. MH acknowledges a PhD scholarship from the ‘Fonds pour la formation à la Recherche dans l’Industrie et dans l’Agriculture’ (FRIA) and the Université catholique de Louvain. We are grateful to Dr. Fred Opperdoes for helpful discussions and critical reading of the manuscript.

References


[49] F.R. Oppedoes, A. Markos, R.F. Steiger, Localization of malate dehydrogenase, adenylate kinase and glycolytic enzymes in glycosomes


