MicroReview

Glycosomes: parasites and the divergence of peroxisomal purpose

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Summary

Peroxisomes are membrane-bounded organelles that compartmentalize a variety of metabolic functions. Perhaps the most divergent peroxisomes known are the glycosomes of trypanosomes and their relatives. The glycolytic pathway of these organisms resides within the glycosome. The development of robust molecular genetic and proteomic approaches coupled with the completion of the genome sequence of the pathogens Trypanosoma brucei, Trypanosoma cruzi, and Leishmania major provides an opportunity to determine the complement of proteins within the glycosome and the function of compartmentation. Studies now suggest that regulation of glycolysis is a strong driving force for maintenance of the glycosome.

Introduction

Trypanosomatids are fascinating and important organisms for two major reasons: their pathogenicity and divergent biology. Among others, trypanosomatids include African trypanosomes (Trypanosoma brucei), American trypanosomes (Trypanosoma cruzi), and Leishmania spp., which cause chronic, often fatal diseases of humans. Vector and animal reservoirs, efficient immune evasion strategies, lack of effective vaccines, poor drugs and emerging resistance make combating these protozoan pathogens an ongoing challenge. The parasites' extensive specialization after divergence from others in the eukaryotic lineage is reflected by numerous unusual features at the genetic, biochemical and cytological level. For example, trypanosomatids extensively decode their mitochondrial transcripts through RNA editing, regenerate thiols using trypaonthione (a glutathione-spermidine conjugate) rather than glutathione, and possess novel cytological structures such as the paralflagellar rod. The unique compartmentation of glycolysis within unusual peroxisomes called glycosomes provides another example of functional divergence. As reviewed here with primary focus on the major human pathogens, the joint results from biochemical analyses, database mining, and molecular genetics are leading to an understanding of the role of glycosomal compartmentation in trypanosomatids.

Glycosomes (Fig. 1), the cytosol, and the mitochondrion cooperate in the energy metabolism of trypanosomatids. The case of the African trypanosome, T. brucei, shows how the different nutritional environments faced during parasite development are reflected in metabolism and organelle function. T. brucei is bathed in constant millimolar levels of glucose as it lives extracellularly in the bloodstream and cerebrospinal fluid of its mammalian host. All ATP is generated through glycolysis and the major proteins in the glycosome are glycolytic enzymes (Hart et al., 1984). In the insect host, conditions change. Tsetse flies feed every few days on blood, their only food. Glucose in the digesting bloodmeal is rapidly metabolized, leaving amino acids as the primary nutrients for the gut-dwelling parasites. Glycosomes remain numerous but most of the glycolytic enzymes decrease in abundance, and other glycosomal enzymes are induced. Oxidative phosphorylation is active, but substrate level phosphorylation is critical to parasite survival (Bochud-Allemann and Schneider, 2002; Coustou et al., 2003). The literature indicates that the carbohydrate metabolism of T. cruzi and Leishmania species probably more closely resembles that of T. brucei procyclic as opposed to bloodstream forms.

What is the basis for calling glycosomes “divergent peroxisomes”? Like peroxisomes, glycosomes are bounded by a single membrane and have a protein-dense matrix. Early studies showed that at least some pathways or enzymes commonly found in peroxisomes are also present in the glycosomes of some species (Oppen, 1984; Wiemer et al., 1996). For example, catalase, the prototypic peroxisomal marker, is found in the glycosomes of the non-pathogenic trypanosomatid Crithidia (Soares and De Souza, 1988). Evidence for a close evolutionary relationship of peroxisomes and glycosomes was bol-
Stereochemistry is the commonality of targeting sequences and unequivocally substantiated by the significant sequence similarity in proteins required for matrix protein import in trypanosomatids, yeast and humans. However, the early finding that most T. brucei glycolytic enzymes are glycosomal (hence the name) established that these organelles are indeed unique. The glycosome may reflect another unusual evolutionary twist: recruitment of proteins from a photosynthetic organism through lateral gene transfer. Whether this gene transfer is a result of endosymbiosis (Hannaert et al., 2003a) or phagocytosis (Waller et al., 2004) remains open. Nonetheless, the signature of the donor is traced in trypanosomatid enzymes and pathways that are related to those of algae, plants and chloroplasts, implying that the genetic partner was an alga (Hannaert et al., 2003a). Interestingly, several plastid-like proteins are present in the glycosome.

How functional glycosomes and peroxisomes are formed

Numerous studies in yeast and humans have shown that a large set of peroxins (PEX) are required for the formation of a functioning peroxisome. Over 20 PEX proteins participate in the import of matrix proteins, and a handful are required for the formation of a functional peroxisomal membrane (including insertion of the membrane proteins) (Purdue and Lazarow, 2001). The first step in import is binding of the peroxisomal targeting sequence (PTS) to a soluble receptor (either PEX5 or PEX7). The receptor-bound cargo docks to a PEX protein complex that allows import. Many PEX proteins are not part of this complex, but are still required for the import of matrix proteins. The mechanism of protein import is truly unique in peroxisomes. The import machinery shows no relationship to those that participate in chloroplast or mitochondrial protein import. Nor do PEX proteins appear related to proteins participating in nuclear or ER import. Moreover, the physical process of import appears to be quite different from chloroplasts or mitochondria, which require protein unfolding. Despite the absence of any apparent pores in the peroxisomal membrane upon electron micrographic analysis, proteins can be imported into the peroxisome fully folded and can even drag along other proteins that lack a targeting sequence (Titorenko et al., 2002)! Indeed, property has been exploited to test for protein–protein interactions: a PTS added to one partner can localize the other partner to the peroxisome (Nilsson et al., 2003). In many organisms, peroxisomal proliferation is controlled by nuclear hormone receptors and transcription factors, often regulated by fatty acids. There is not any evidence for this type of regulation of glycosomes in trypanosomatids.

Several years ago, our laboratory used a genetic screen to identify an L. donovani gene involved in glycosome biogenesis. To our satisfaction, the encoded protein showed low, albeit significant, similarity to yeast and human PEX2 (Flaspohler et al., 1997), which is required for peroxisomal protein import. Since then, several other PEX homologues have been identified in African trypanosomes and several species of Leishmania. In most cases the level of sequence identity with PEX proteins in other eukaryotes is rather low and conclusive identification has required corroborating evidence such as localization to the glycosome. In contrast, once a PEX gene has been identified in one trypanosomatid, the homologue in other trypanosomatids can be readily identified from the genomic sequence. The involvement of several PEX homologues in glycosomal function has been directly demonstrated (e.g. Lorenz et al., 1998; Furuya et al., 2002; Guerra-Giraldez et al., 2002). As discussed below, the identification of PEX genes and proteins, together with advances in genetic technologies in trypanosomatids, provides an opportunity to assess the functional importance of the glycosome.

Peroxisomal and glycosomal metabolic pathways

Experimental biology has elucidated much of what we know about the metabolic pathways contained in peroxisomes and glycosomes, but database mining and sequence analysis have recently suggested the organellar location of additional proteins and pathways. A study examining eight eukaryotic genomes ranging from yeast to humans identified 430 predicted proteins bearing a probable type 1 PTS (Emanuelsson et al., 2003). Database analysis searching for proteins bearing the less well-defined PTS2 will undoubtedly identify numerous additional candidate peroxisomal proteins. The presence of a PTS does not ensure that the protein is predominantly glycosomal. For example, trypanothione reductase terminates in a PTS1, but is predominantly cytosolic (Smith et al., 1991). Furthermore, not all known peroxisomal/glycosomal proteins bear a recognizable PTS. Hence, data-
base analysis must be supplemented with experimental biology to identify peroxisomal/glycosomal proteins. The stage-specific metabolism described above means that a full understanding of glycosomal metabolism requires an analysis of expression in addition to sequences. Proteomics offers an opportunity to identify candidate glycosomal proteins and to examine their expression during development. This technology has been applied to peroxisomes of plants, yeast and animals, allowing the identification of novel proteins, including those likely to function in regulation such as protein kinases (Fukao et al., 2002). The completion of trypanosomatid genome sequences coupled with proteomics may allow a definition of the full constellation of glycosomal proteins in the near future.

One theme that has emerged over the years is that peroxisomes are organelles whose specialization reflects the peculiarities of each species. While in most species, peroxisomes compartmentalize ether-lipid synthesis and β-oxidation of fatty acids, in other species they additionally compartmentalize some steps of isoprenoid synthesis, aromatic amino acid synthesis, purine catabolism or other oxidative pathways. Similarly, glycosomes contain many of the hallmark peroxisomal activities, plus specialized processes. At this juncture, there is little data to suggest major differences in the complement of enzyme pathways contained in glycosomes of different trypanosomatids. Like peroxisomes, T. brucei glycosomes contain several enzymes in pathways of ether-lipid biosynthesis (dihydroxyacetone-phosphate acyltransferase, 1-acyl glycerol-3-phosphate: NADP oxidoreductase, and acyl-coA reductase) (Opperdoes, 1984) and at least two activities of β-oxidation (2-enoyl coA hydratase and hydroxyacyl-dehydrogenase, encompassed in a single protein) (Wiemer et al., 1996). The presence of a PTS-1 on T. brucei and T. cruzi carnitine acetyl transferase, catalysing the last peroxisomal step in fatty acid oxidation (it is required for the transmembrane transport of the product, acetyl coA), suggests that the full peroxisomal pathway could be present in glycosomes at some stage of development. Although mammals possess specialized fatty acid oxidation systems in mitochondria and peroxisomes, little oxidation of fatty acids was observed in the mitochondrial fraction of T. brucei (Wiemer et al., 1996). These data suggest that the glycosomal pathway is required for utilization of fatty acids as a carbon and energy source. Although Leishmania mexicana amastigotes show higher activity than promastigotes (Coombs et al., 1982), the importance of this pathway to parasite survival remains unclear.

Peroxisomal β-oxidation generates hydrogen peroxide, which is broken down by peroxisomal catalase. Interestingly, this enzyme is absent in T. brucei and Leishmania. However, T. cruzi possesses a glycosomal glutathione-dependent peroxidase that could remove hydrogen peroxide (Wilkinson et al., 2002), and the T. brucei predicted protein contains a PTS1 (Hillebrand et al., 2003). A subset of Leishmania chagasi superoxide dismutases are also reported to be glycosomal (Plewes et al., 2003).

A few reports suggest that the pentose phosphate pathway is peroxisomal in some organisms. In T. brucei, this pathway provides an important example of dual localization effected by partial targeting. Single copy genes encode the enzymes, but at least the first activities of the pathway (glucose-6-phosphate dehydrogenase and 6-phosphogluconolactonase) are partitioned between the glycosome and cytosol (Duffieux et al., 2000). Several enzymes which catalyse subsequent steps bear PTS1 sequences. Barrett suggested that the cytosolic pathway may fulfill both reductive and biosynthetic functions (phosphorylated sugars for nucleotide salvage and biosynthesis and the building blocks for aromatic amino acid synthesis), while the glycosomal pathway may primarily serve to regenerate reducing equivalents (Barrett, 1997). The recent finding of some purine salvage enzymes in the glycosome (see below) broadens this view, because the glycosomal pentose phosphate pathway would produce the ribose-5-phosphate required for the salvage reactions. In any case, the NADPH regenerated by the pentose phosphate pathway can be used in biosynthetic reactions or to reduce glutathione (or trypanothione) to combat oxidative stress. Among the more surprising proteins that may be associated with this pathway is a Calvin cycle enzyme heretofore considered to be plant-specific: sedoheptulose-1,7-bisphosphatase. The T. brucei predicted protein bears a PTS1 and may be a relict of an ancient endosymbiosis (Hannaert et al., 2003a) or perhaps an algal lunch (Waller et al., 2004).

The localization of the trypanosomatid enzymes involved in sterol and isoprenoid metabolism have been little studied, although some of them bear predicted PTSs. The molecular signals for targeting membrane proteins to peroxisomes are not well-defined. The low permeability of peroxisomes to small molecules such as NAD/NADH, ATP, and acetyl coA, as deduced from studies of yeast peroxisomes (Van Roermund et al., 1995) clearly indicate that specific carriers are required to transport substrates and products across the organelle membrane. Some transporters have been defined at the molecular level for peroxisomes, such as those involved in uptake of activated fatty acids, but they are not yet known for glycosomes. The low permeability for small molecules also indicates that the organelle must contain activities to regenerate cofactors and ATP so that metabolism can continue.

**Pathways unique to glycosomes**

The most unusual pathway detected in glycosomes is the
Embden–Meyerhof segment of glycolysis (Fig. 2). Glucose is imported into the parasite using specific transporters (often stage-specific) and then into the glycosomes via an unknown mechanism. The enzymes from hexokinase to phosphoglycerate kinase (or glyceraldehyde-3-phosphate dehydrogenase in the case of T. brucei procyclic forms) are present in glycosomes. Within the glycosome, no net synthesis ATP occurs as a result of glycolysis. Rather ATP is generated outside of the glycosome, when pyruvate kinase transfers the high energy phosphate from phosphoenolpyruvate to ADP.

Glycosomal glycolysis requires the regeneration of NAD⁺, which is accomplished through a collaboration with the mitochondrial glycerol phosphate oxidase complex in bloodstream forms. As shown in Fig. 2 (green arrows), glycosomal glyceraldehyde phosphate dehydrogenase generates glyceraldehyde-3-phosphate and NAD⁺. The glyceraldehyde-3-phosphate is reoxidized by the mitochondrial glyceraldehyde phosphate complex [which is comprised of glyceraldehyde phosphate dehydrogenase (FAD), a plant-like alternative oxidase, and ubiquinone], and returned to the glycosome for further cycling. Without such a cycle, glyceraldehyde-3-phosphate could not be diverted to glyceraldehyde-3-phosphate, sacrificing half of the net ATP synthesis per glucose. When this shuttle is disrupted through biochemical or genetic means, T. brucei bloodstream forms die because the glycolytic pathway is blocked.

Preliminary database mining has recently revealed the presence of a key enzyme for gluconeogenesis in T. brucei: fructose-1,6-bisphosphatase (Hannaert et al., 2003b). The predicted protein contains both a PTS1 and PTS2, strongly suggesting that it is localized to glycosomes. Several other glycolytic enzymes could participate in gluconeogenesis, functioning in reverse. It is unclear how metabolism is regulated such that the two pathways do not result in futile cycling. A few other carbohydrate metabolizing enzymes are present in the glycosomes, with their primary function possibly being the recovery of oxidizing equivalents to spur glycolysis and perhaps β-oxidation of fatty acids.

Purine salvage occurs in glycosomes. Because the parasites do not synthesize purines de novo, the enzymes are of considerable importance. L. donovani hypoxanthine guanine phosphoribosyl transferase and xanthine phosphoribosyl transferase both contain PTS1 signals and have been localized to glycosomes (Zarella-Boitz et al., 2004). Adenine phosphoribosyl transferase does not contain a recognizable PTS. The presence of these salvage enzymes in glycosomes is a mystery because they are cytosolic in other organisms. Xanthine phosphoribosyl
transferase is functional for purine salvage when expressed in the cytosol (Zarella-Boitz et al., 2004). A few steps of pyrimidine metabolism, typically cytosolic in other organisms, also occur in glycosomes. Orotate phosphoriboisyltransferase and orotidine-5'-phosphate decarboxylase activities are mediated by a fusion protein bearing a PTS1 (Gao et al., 1999).

The role of peroxisomal compartmentation

The disorders of peroxisome biogenesis in humans provide some insights as to the consequences of loss of compartmentation. Although affected individuals survive to birth and their cells are viable in culture, those with the most severe disease, Zellweger’s syndrome, die in infancy. These patients are deficient in oxidation of long-chain fatty acids and cannot use them as carbon and energy sources. Long-chain and very-long-chain fatty acids accumulate and may be toxic. Similarly, yeast mutants defective in peroxisome biogenesis cannot grow on fatty acids, although they are healthy when grown on other substrates. Zellweger’s cells are also defective in ether-lipid synthesis. Defects in lipid metabolism may alter the levels of small molecule mediators that affect the development of the nervous system and possibly other processes. These data suggest that disruption of glycosomal biogenesis would likely result in the abrogation of these pathways, eliminating the ability to use fatty acids as carbon and energy sources and changing the cellular lipid profile.

The function of glycosomal compartmentation

Unlike the peroxisomes of yeast and humans, the glycosomes of T. brucei are essential for cellular survival. RNAi-mediated knockdown of PEX2 (Guerra-Giraldez et al., 2002) or PEX14 (Furuya et al., 2002) disrupts import of matrix proteins into the glycosome and kills both mammalian and insect stages. The inability to obtain PEX gene knockouts in Leishmania suggests that glycosomes are essential in this genus as well. We propose that as a result of the evolutionary history of the glycosome, multiple factors drive its maintenance.

Perhaps most obviously, the lack of glycosomes could result in deficiencies of enzymes that are important at some particular stage of the life cycle. Here one would suspect the culprits to be enzymes of ether-lipid synthesis or β-oxidation of fatty acids, because compartmentation is required to prevent cytosolic degradation of these enzymes in other organisms. We are unaware of any tests of the requirement for oxidation of fatty acids in trypansomatids. However, recently the role of ether-lipid biosynthesis was explored in L. major. In Leishmania promastigotes, the major surface glycoconjugate, lipophosphoglycan (LPG), is GPI-anchored through an ether lipid. Surprisingly, genetic blockade of ether-lipid formation does not affect the viability of promastigotes, even though LPG and several other major glycoconjugates are absent (Zufferey et al., 2003). However, the lack of ether lipids strongly impairs the ability of the parasites to transit the initial phases of macrophage infection. Once infection was established and amastigotes formed, these parasites retained their virulence. We propose that glycosomal compartmentation is required for biosynthesis of parasite ether lipids needed for effectively completing the Leishmania life cycle. With respect to the roles of ether lipids in other parasites, less is known. The major surface glycoconjugates of T. brucei are GPI-anchored through acyl lipids.

Glycolysis may be the most important driving force for maintenance of the glycosome. Early hypotheses suggested that compartmentation might provide for a higher efficiency of glycolysis through maintaining a high concentration of metabolites. However, comparison with Baker’s yeast indicates that compartmentation is not required for a high glycolytic flux (Bakker et al., 2000; Hannaert et al., 2003a). Recent data suggest that compartmentation may be most important in preventing metabolic interference. Because of the low permeability of the peroxisomal (glycosomal) membrane, enzymes and metabolites in the cytosol and glycosome are segregated from one another. Expression of some glycolytic enzymes in the cytosol is toxic. For example, phosphoglycerate kinase is exclusively glycosomal in T. brucei bloodstream forms, and when it is expressed in the cytosol, the parasites die (Blattner et al., 1998). Because this experiment was performed on cells co-expressing wild-type levels of the glycosomal form, the glycosomal glycolytic pathway was still intact. These findings suggest that the mislocalized enzyme might be consuming cytosolic substrates or generating products at levels the cell cannot tolerate. Similarly, triose phosphate isomerase expressed in the cytosol is toxic to bloodstream forms (Helfert et al., 2001). This toxicity could result from the disruption of the glyceraldehyde-phosphate shunt.

Regulation by glycosomal compartmentation

Some catabolic pathways require an initial investment of ATP so that the following reactions, which generate ATP, can be thermodynamically favourable. Glycolysis is one of those pathways. Described by Westerhoff and colleagues as a ‘turbo design’, this early investment requires that glycolysis be kept under control (Teusink et al., 1998). In most organisms, control is accomplished by tightly regulating the early ATP utilizing steps catalysed by hexokinase and phosphofructokinase. However, purified hexokinase and phosphofructokinase from trypanosoma-
The mechanism of glucose toxicity in the absence of glycosomes in procyclic forms remains to be experimentally elucidated, although the findings are compatible with the computer model of bloodstream forms described above. From that model, one would predict that knockdown of hexokinase, required for entry of glucose into the glycolytic pathway, would suppress the effects of PEX14 RNAi. Such experiments are underway in our laboratory. Substrate-accelerated death has also been observed in mutant yeast lacking trehalose-6-phosphate synthase, a regulator of glycolysis. When glucose is omitted, the yeast survive. Furthermore, downregulation of hexokinase activity was able to suppress lethality (Hohmann et al., 1993), compatible with a kinetic model showing that fivefold reduction alleviates toxicity (Teusink et al., 1998).

In procyclic forms, hexokinase is naturally downregulated over 10-fold as compared to the levels found in bloodstream forms (Hart et al., 1984), which were used to generate the kinetic model.

More effort will be required to determine whether death is because of the generation of toxic levels of phosphorylated intermediates and consumption of ATP or a more complex mechanism. Measurements of the level of glycolytic intermediates could be helpful in this regard, although discounting other effects may be difficult. For example, it has been shown that removal of glucose or knockdown of hexokinase results in an alteration of the surface protein profile of procyclic form parasites (Morris et al., 2002). Hence, some metabolite of glucose may play an important regulatory role in the parasite life cycle. The dysregulation caused by disruption of glycosomal compartmentation could be more far-reaching than osmotic effects mediated by the accumulation of intermediates.

Could glycosomal compartmentation constrain other processes? Most other reactions that occur in the glycosome do not require glycosomal ATP. Even though β-oxidation of fatty acids requires an investment of ATP, this investment occurs in the cytosol. Whether glycosomal ATP is required to maintain other glycosomal pathways requires further examination. Another possibility is that compartmentation allows a different ratio or concentration of NAD and NADH to be maintained within the glycosome, thereby imposing an additional level of control in the organelle. Additionally, it will be important to expand studies from T. brucei to examine the role of the glycosome in the mammalian stages of T. cruzi and Leishmania.

Trypanosomatids cause some of the most intractable diseases of the world (African sleeping sickness, Chagas' disease and Leishmaniasis), and are also among the most cytologically and biochemically unique eukaryotes. Several unusual features of the parasite are related to their energy metabolism: the glycosome, RNA editing, plant-like pathways and enzymes. Could these features represent a target for attacking the parasite? For T. brucei, blocking glycolysis would be toxic in the mammalian stage. Although the parasites' glycolytic enzymes closely resemble those of the host (despite differences in regulation), some trypanosomatid-specific compounds have been synthesized based on crystal structures and have been shown to block parasite growth (Verlinde et al., 2001). More speculatively, compounds that disrupt the localization of proteins to the glycosome should also be toxic. Blocking some step in the cascade of protein interaction events required for matrix protein import could be
a means to this end. Although the challenges are considerable, recent progress in developing paradigms for the identification of small molecules that modulate protein interactions encourages further studies towards this goal (Berg, 2003; Gadek and Nicholas, 2003).

Acknowledgements

Dr Sanjiban Banerjee kindly provided the immunofluorescence image in Fig. 1. The author thanks Dr Paul Michels for helpful discussions, and Drs Peter Kessler, Amy DeRocher and Bryan Jensen for critical reading of the manuscript. This work was supported in part by NIH R01 AI22635.

Reference


