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Review

Acquisition and biosynthesis of saturated and unsaturated fatty acids by trypanosomatids

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ABSTRACT

As components of phospholipids and glycosylphosphatidylinositol anchors, fatty acids are responsible for forming the core of biological membranes and the correct localization of proteins within membranes. They also contribute to anchoring proteins by direct acylation of specific amino acids. Fatty acids can be used as energy sources and serve as signaling molecules or precursors for their synthesis. All these processes highlight the important role of fatty acids in cell physiology, justifying the diverse strategies for their acquisition evolved by different organisms. This review describes several recent findings in the salvage and biosynthesis of fatty acids by parasitic protists belonging to the class Kinetoplastea. They include two biosynthetic routes, the mitochondrial one and a peculiar membrane-associated pathway, the synthesis of polyunsaturated fatty acids, and the scavenging of lysophospholipids and lipoproteins from host plasma. These different processes are also explored as putative targets for chemotherapy.

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1. Introduction

Protists belonging to the Trypanosomatidae family, such as *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania* spp., are parasitic flagellates responsible for several human and animal diseases, like Chagas' disease, sleeping sickness and leishmaniases. Together, they threaten over 400 million people, with 30 million currently being infected, mainly distributed in underdeveloped countries. These neglected diseases are often fatal if not treated [1].

For years, trypanosomatids have been considered as a source of rarities, having a number of diverse, unusual biological features like aerobic fermentation, with glycolysis compartmentalized in organelles named glycosomes [2], editing of mitochondrially encoded pre-mRNAs, transsplicing of nuclear transcripts and antigenic variation [3]. These peculiarities were attributed to the supposed early evolutionary branching of these organisms from the main trunk of the eukaryotic tree, considering them as primitive characters. Current opinion holds that this last concept is most probably incorrect; most of these processes are more easily explained by considering them as acquisitions by which the ancestral kinetoplastids adapted to their environment and which were elaborated when later these flagellates adapted parasitic life styles.

Fatty acid (FA) acquisition by trypanosomatids can equally be considered as a process with peculiarities that resulted from adaptation to the parasitic conditions. In the 1970s it was found that trypanosomatids had an apparently low or even null capacity for *de novo* FA biosynthesis, which was in agreement with the metabolic

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dependence expected for parasites. Interestingly, instead of losing the biosynthetic capacity, the parasites had evolved new strategies to take up lipids from their environmental milieu as well as established a novel metabolic pathway for *de novo* synthesis of FAs. The nature of this pathway, only recently elucidated in its unexpected details, had provoked decades of disconcert in the scientific community. As the features of fatty acid biosynthesis in the trypanosomatids are different enough from those present in mammals, they reveal potential for development of antiparasitic drugs.

This review highlights the recent findings related to the scavenging and synthesis of FAs by trypanosomatids. Excellent reviews have previously been published by others, dealing with the acquisition of sterols [4], sphingolipids [5,6], phospholipids [5,7–9] and FAs [4,9,10]. Finally, I hypothesize about the origin of precursors for FA synthesis, based on recent findings about the catabolism of energy sources and analysis of the information available in trypanosomatid genome databases.

2. Scavenging of lipids as fatty acid source

Trypanosomatids have evolved multiple ways to scavenge lipids from their hosts. Plasma lipoproteins like LDL represent a main source of sterols and phospholipids. These parasites are able to internalize lipoproteins by receptor-mediated endocytosis [11–13]. Once endocytosed, the LDL receptor is recycled and LDL-derived apolipoprotein B degraded in lysosomes, releasing steryl esters and phospholipids. These lipids are then hydrolyzed, providing FAs, sterols and phospholipid head groups to the cell (Fig. 1) [14,15]. Another important source of lipids are HDL particles, which are also receptor-mediated endocytosed by *T. brucei* [16]. Interestingly, a subtype of human HDL is lytic for the *T. brucei brucei* subspecies [17], whereas *T. b. rhodesiense* and *T. b. gambiense* have developed resistance mechanisms against its trypanolytic factors [18,19].

Bloodstream forms of *T. brucei* have an additional way for acquiring FAs and phospholipids head groups by scavenging the relatively abundant lysophosphatidylcholine, which is present at hundreds of micromolar concentrations in host plasma [20]. It is performed through a pathway consisting of three enzymes, phospholipase A1, acyl-CoA synthetase and lysophosphatidylcholine:acyl CoA acyltransferase [21]. Phospholipase A1 generates FAs from exogenous lysophosphatidylcholine which are channeled to the synthetase, producing acyl-CoA. The acyl-CoA formed by this route is in a separate pool from that derived from exogenous free FAs and could be alternatively used by the acyltransferase to acylate a second molecule of exogenous lysophospholipid, providing phosphatidylcholine to the cell (Fig. 1).

Although free FAs are present in low amounts in plasma [22], they are readily taken up by kinetoplastid protists [23,24]. The uptake presents a biphasic course, with an initial rapid phase. Depending on the FA and parasite species, 50–90% of FA incorporation occurs during the first 15 s of the radiolabeling experiment. It is followed by a slower accumulation phase. The initial phase is thought to represent the binding of FAs to the cell surface, which is mediated by serum albumin in a reversible process (Fig. 1). Supporting this hypothesis, the release of preloaded FAs from *T. brucei* cells is stimulated by a direct interaction with free albumin [23]. In mammalian cells, the binding of FA to the cell surface is assisted by the plasma membrane FA binding protein (FABPpm) and glycoprotein CD36. Whereas there are no obvious orthologs of CD36 in the trypanosomatid' genome, FABPpm shows high amino acid identity to kinetoplastid' mitochondrial aspartate aminotransferases. FABPpm has a dual function and localization in mammalian cells. It localizes at the mitochondria having aspartate aminotransferase activity as well. In addition, FABPpm was also found on the cell surface of several tissues [22]. Whether the corresponding trypanosomatid proteins have a dual localization or are involved in FA uptake is not known.

Once at the cell surface, FAs can cross the plasma membrane by simple (passive) diffusion, or be translocated by membrane-associated FA transport proteins (FATPs, Fig. 1) [22,25]. In a similarity search using yeast Fat1p or human FATP1 amino-acid sequences as queries, we identified orthologs in the genome of *T. cruzi* (TriTrypDB identification number TcCLB.511907.110) and *Leishmania major* (LmjF.24.1780), but not in that of *T. brucei* (Table 1). These are large proteins (1064 and 1311 amino acids, respectively) compared to Fat1p (623 amino acids) but share important similarities, particularly in the region supposed to be involved in FA recognition. This difference between *T. brucei* and other trypanosomatids is intriguing, but could be related to the extracellular condition of the former species, while the other parasites live predominantly intracellularly in mammals. If the proteins identified above are *bona fide* translocators, it could be possible that they are needed in the uptake of free FAs which are found in very low amounts within the host cell, whereas *T. brucei* replaced FATPs by a system to more efficiently rely on the relatively abundant lysophospholipids found in plasma (see above). Another possibility is that FATPs are involved in the uptake of very long chain FAs (VLFAs, Fig. 1), which are used in the synthesis of glycosylphosphatidylinositol (GPI) anchors of *T. cruzi* and *Leishmania* spp. but not in *T. brucei* GPI [10]. Although the former parasites are able to *de novo* synthesize C24 and C26 VLFAs (see Section 3), the uptake would contribute to assure enough VLFAs for the correct localization of essential macromolecules on the cell surface *via* GPI anchoring.

Internalized FAs are activated to their CoA thioesters by acyl-CoA synthetases (ACS), trapping them inside the cell. This acylation represents a vectorial transport of FAs which are then available for subsequent metabolism [22,25]. Trypanosomatids have numerous putative ACSs. *T. brucei* has five synthetases (ACS1–5) [7,26] sharing 26–28% identity with the yeast ACS FAA1 and other two putative ones (Tb927.11.4490 and Tb927.11.7530, Table 1) distantly related (less than 15% identity). TbACS1–4 are encoded on chromosome 9 in a tandem array, whereas TbACS5 is encoded on chromosome 10. TbACS1 showed preference for polyunsaturated fatty acids of 18–22 carbons (C18–22 PUFAs) and C10–14 saturated FAs [26]. TbACS2 was only active on short saturated FAs (C6–12), whereas TbACS3–4 were active on saturated C8–18 FAs as well as unsaturated C16–20 FAs [26]. TbACS5 showed preference for the saturated FA myristic acid (14:0) [7]. They usually are membrane associated proteins but their subcellular localization in these parasites was not determined. It is not known which of these ACSs is involved in the scavenging of lysophosphatidylcholine described above. *Saccharomyces cerevisiae* internalizes PUFAs inefficiently, but the expression of TbACS1 stimulated two- to sixfold the incorporation of exogenous C20–22 PUFAs into the yeast [Tripodi and Uttaro, unpublished results]. TbACS1was potentially expressed at the plasma membrane, interacting with endogenous yeast FATP (Fat1p), and then mediating a vectorial transport. Co-expression of TbACS1 and trypanosomatid FA desaturases and elongases (Sections 3 and 4), allowed us to notably increase these heterologous activities using exogenous FA substrates, facilitating their biochemical characterization. It is expected that equivalent interactions between ACSs and FATPs also occur in trypanosomatids.

Acyl-CoAs usually bind to proteins (acyl-CoA-binding proteins or ACBPs) to prevent their metabolism as free acyl-CoAs and allow their efficient shuttling to the cell acylation machinery. An ACBP (Table 1) was isolated and characterized in *T. brucei*, which showed preference for saturated C10-14 acyl-CoAs. Its presence stimulated the incorporation of myristate from myristoyl-CoA into GPI, but not into neutral lipids or phospholipids, in a cell-free assay [27].

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