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The glycosomal alkyl-dihydroxyacetonephosphate synthase *Tb*ADS is essential for the synthesis of ether glycerophospholipids in procyclic trypanosomes

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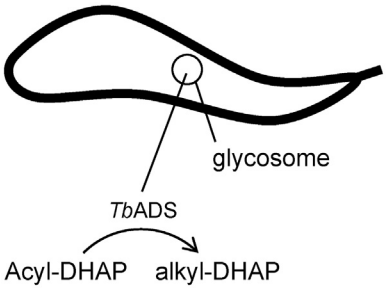
HIGHLIGHTS

- *Tb*ADS exhibits alkyl-dihydroxyacetonephosphate synthase activity.
- *Tb*ADS is important for ether lipid biosynthesis in procyclic trypanosomes.
- *Tb*ADS localizes to the glycosomal lumen.
- *Tb*ADS is dispensable for procyclic trypanosomes' viability.

GRAPHICAL ABSTRACT



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ABSTRACT

Glycerophospholipids are the main constituents of the biological membranes in *Trypanosoma brucei*, which causes sleeping sickness in humans. The present work reports the characterization of the alkyl-dihydroxyacetonephosphate synthase *Tb*ADS that catalyzes the committed step in ether glycerophospholipid biosynthesis. *Tb*ADS localizes to the glycosomal lumen. *TbADS* complemented a null mutant of *Leishmania major* lacking alkyl-dihydroxyacetonephosphate synthase activity and restored the formation of normal form of the ether lipid based virulence factor lipophosphoglycan. Despite lacking alkyl-dihydroxyacetonephosphate synthase activity, a null mutant of *TbADS* in procyclic trypanosomes remained viable and exhibited normal growth. Comprehensive analysis of cellular glycerophospholipids showed that *Tb*ADS was involved in the biosynthesis of all ether glycerophospholipid species, primarily found in the PE and PC classes.

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1. Introduction

Trypanosoma brucei is a flagellated protozoan parasite of the Kinetoplastida class that causes the diseases sleeping sickness in humans and nagana in cattle in Africa. The life cycle of this unicellular microbe comprises an insect stage, where it develops in the digestive system and salivary glands of the tsetse fly vector followed by a vertebrate host stage, where it multiplies in the bloodstream of an infected mammal.

Abbreviations: ADS, alkyl-dihydroxyacetonephosphate synthase; DHAP, dihydroxyacetonephosphate; DHAPAT, DHAP acyltransferase; ESI-MS/MS, electrospray ionization tandem mass spectrometry; G3P, glycerol-3-phosphate.

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Glycerophospholipids are the most abundant lipids in *T. brucei*, representing 80% of total cellular lipids (Smith and Butikofer, Patnaik et al., 1993; Smith and Butikofer, 2010). The five main classes are phosphatidylcholine (PC, 45–60%), phosphatidylethanolamine (PE, 10–20%), phosphatidylinositol (PI, 6–12%), phosphatidylserine (PS, <4%), and cardiolipin (<3%; reviewed in (Smith and Butikofer, 2010; Serricchio and Butikofer, 2011; Farine and Butikofer, 2013; Ramakrishnan et al., 2013). While most of the glycerophospholipids are ester glycerophospholipids that bear a fatty acid at position one of the glycerol backbone, a significant proportion of glycerophospholipids (mainly PE and PC species) in this parasite carry an ether linked aliphatic fatty alcohol (ether glycerophospholipids) instead (Patnaik et al., 1993; Richmond et al., 2010); reviewed in (Smith and Butikofer, 2010)). Beside their structural function as the main constituent of the biological membrane, glycerophospholipids are also involved in various essential biological processes. As second messengers of signaling pathways, they regulate key processes such as membrane trafficking, cell cycle progression, and dynamic of organelles such as mitochondria and endoplasmic reticulum (Coppolino et al., 2002; Krauss et al., 2006; Santarius et al., 2006; Gibellini et al., 2009; Signorell et al., 2009; Zhang et al., 2009; Serricchio and Butikofer, 2011; Farine and Butikofer, 2013; Ramakrishnan et al., 2013). Glycerophospholipid-based molecules have also been shown to be implicated in virulence in *Leishmania* and *T. brucei* (Spath et al., 2000; Turco et al., 2001; Spath et al., 2003; Zufferey et al., 2003; Ponte-Sucre, 2016).

Ether glycerophospholipid biosynthesis initiates with the acylation of DHAP by a dihydroxyacetonephosphate (DHAP) acyltransferase (DHAPAT) to yield acyl-DHAP. The latter is then converted to 1-alkyl-DHAP by an alkyl-DHAP synthase (ADS), which removes the acyl group and replaces it with a fatty alcohol, thus introducing the ether linkage. The alkyl-DHAP synthase catalyzes the committed step as its product, 1-alkyl-DHAP, serves as the obligate precursor for the production of all ether glycerophospholipids. Then, an NADPH dependent alkyl/acyl-DHAP reductase converts 1-alkyl-DHAP to 1-alkyl-glycerol-3-phosphate (1-alkyl-G3P). The DHAPAT enzyme can also contribute to the production of ester glycerophospholipids when its product, 1-acyl-DHAP, is directly reduced to 1-acyl-G3P by the acyl/alkyl-DHAP reductase (Jones and Hajra, 1983; Liu et al., 2005). All three enzymes are associated with the peroxisomes. In *T. brucei*, DHAPAT activity is mediated by two enzymes, *Tb*DAT and to a lesser extent, *Tb*GAT (Fig. 1; Patel et al., 2016; Zufferey et al., 2017). *Tb*DAT and *Tb*GAT exhibit slightly different specificities regarding the fatty acyl-CoA donor and localize in different subcellular compartments; *Tb*GAT resides in the endoplasmic reticulum, while *Tb*DAT localizes to peroxisome-related organelles, called glycosomes in protozoan parasites of the family Trypanosomatidae (Opperdoes, 1984; Zomer et al., 1995; Heise and Opperdoes, 1997; Vertommen et al., 2008; Patel et al., 2016). Both enzymes are dispensable for normal growth, but only *Tb*DAT is important for ether glycerophospholipid biogenesis.

Despite their lower abundance compared to that of ester glycerophospholipids, ether glycerophospholipids fulfill essential cellular functions (reviewed in (Gorgas et al., 2006)). In mammals, they play an important role in membrane trafficking, in the release and composition of exosomes, and in angiogenesis for retina development (Thai et al., 2001; Saab et al., 2014; Phuyal et al., 2015). In the neuronal system, they regulate exocytosis and efflux of neurotransmitters in synapses, and play an important role in myelination (Teigler et al., 2009; Brodde et al., 2012). Furthermore, ether glycerophospholipids are essential for spermatogenesis and development of the optic nerve (Rodemer et al., 2003). In *T. brucei*, ether PE have been shown to be important for the integrity of the inner membrane of the mitochondria and completion of cytokinesis/cell division (Signorell et al., 2008; Signorell et al., 2009; Farine and Butikofer, 2013).

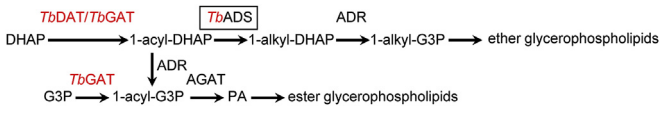

Fig. 1. Putative glycerophospholipid biosynthetic pathways of *T. brucei*. AGAT, 1-acyl-G3P acyltransferase; ADR, acyl/alkyl-DHAP reductase; PA, phosphatidic acid.

ADS enzymes from several diverse living organisms have been characterized genetically and biochemically (reviewed in (van den Bosch and de Vet, 1997)). ADS is a luminal, surface peroxisomal membrane associated enzyme that utilizes a FAD cofactor to exchange the acyl with an alkyl group (Hardeman and van den Bosch, 1989, Zomer et al., 1993; Zomer et al., 1995; de Vet et al., 1997; de Vet et al., 2000; Razeto et al., 2007; Nenci et al., 2012). In higher eukaryotes, lack of ADS leads to cataract resulting from defective lens fiber cells, and male sterility due to impaired sperm biogenesis (Liegel et al., 2011; Liegel et al., 2014). Human mutations in the *ADS* gene are responsible for a pathological condition called rhizomelic chondrodysplasia punctata form 3, which is characterized by skeletal dysplasia and mental retardation (Itzkovitz et al., 2012; Noguchi et al., 2014). Increased ADS activity, and consequently higher levels of ether glycerophospholipids levels, has been associated with cancer; conversely, ablation or depletion of *ADS* resulted in reduced cell survival and proliferation, and decreased cancer aggressiveness and tumor growth (Lee et al., 1980; Benjamin et al., 2013; Zhu et al., 2014a; Zhu et al., 2014b). The ADS enzyme is also important for virulence in the protozoan parasite *Leishmania major*, as the ether glycerophospholipid based virulence factor lipophosphoglycan was impaired in a mutant strain lacking this enzyme (Zufferey et al., 2003). In *T. brucei*, the alkyl-DHAP synthase activity was characterized and its gene was cloned (Zomer et al., 1995; Zomer et al., 1999). However, its role in parasite's biology was not investigated yet. The present work focuses on the biochemical characterization and role of the ether glycerophospholipid committed enzyme *Tb*ADS in growth and lipid metabolism of procyclic trypanosomes.

2. Material and methods

2.1. Strains and growth conditions

The wild-type strain of procyclic forms of *T. brucei* used in this work is stock 427-60 and was cultivated in SDM-79 medium supplemented with 10% heat inactivated fetal bovine serum as described in (Brun and Schonenberger, 1979). Growth, transformation, and limiting dilution of parasites were carried out as previously described (Patel et al., 2016). Transgenic lines were selected in the presence of 5 μg/ml blasticidin, 2 μg/ml puromycin, and 3 μg/ml phleomycin as appropriate. Growth curves were performed by seeding the culture at a cell density of 1×10^6 cells/ml. Parasites were counted with a hemocytometer as a function of time.

Promastigotes of *L. major* Friedlin V1 strain (MHOM/IL/80/Friedlin) were propagated in liquid M199-based medium (Zufferey et al., 2003). The null mutant *Δlmads/Δlmads* and complemented line *Δlmads/Δlmads/LmADS* were described previously (Zufferey et al., 2003). Transfection was performed according to Ngo and colleagues, and selection was accomplished in the presence of 50 μg/ml puromycin (Ngo et al., 1998).

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