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The ins and outs of phosphatidylethanolamine synthesis in *Trypanosoma brucei*☆

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ABSTRACT

Phospholipids are not only major building blocks of biological membranes but fulfill a wide range of critical functions that are often widely unrecognized. In this review, we focus on phosphatidylethanolamine, a major glycerophospholipid class in eukaryotes and bacteria, which is involved in many unexpected biological processes. We describe (i) the ins, i.e. the substrate sources and biochemical reactions involved in phosphatidylethanolamine synthesis, and (ii) the outs, i.e. the different roles of phosphatidylethanolamine and its involvement in various cellular events. We discuss how the protozoan parasite, *Trypanosoma brucei*, has contributed and may contribute in the future as eukaryotic model organism to our understanding of phosphatidylethanolamine homeostasis. This article is part of a Special Issue entitled Phospholipids and Phospholipid Metabolism.

1. Introduction

1.1. Phosphatidylethanolamine

Phospholipids represent major components of all biological membranes and comprise of two categories: (i) the glycerophospholipids, which are composed of a hydrophilic head (neutral or charged) linked via a phosphate group to a glycerol moiety carrying fatty acyl or alcohol chains, and (ii) the sphingophospholipids, in which the phosphate-linked head groups are attached to ceramide. The major classes among glycerophospholipids include phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylinositol (PI), whereas the major sphingophospholipid classes comprise of sphingomyelin (SM), inositolphosphoryl ceramide (IPC) and ethanolaminephosphoryl ceramide (EPC). Their relative abundances vary greatly among organisms, tissues, cell types and organelles [1]. In addition, phospholipids are distributed asymmetrically between the two leaflets of lipid bilayers: in eukaryotic cells, PS is found exclusively, and PE and PI predominantly, in the cytoplasmic leaflet of the plasma membrane, whereas the choline-containing phospholipid classes, PC and SM, are enriched in the outer leaflet [2–4]. This asymmetric distribution of phospholipids is generated and maintained by bi-directional transport mechanisms involving ATP-independent and ATP-dependent lipid flippases in the endoplasmic reticulum and the plasma membrane, respectively [5,6]. The glycerophospholipids of Archaea, Bacteria and Eukarya differ in the composition of the hydrophobic tails and, most notably, the stereochemistry of the glycerophosphate moiety, but in general share the same head groups [7]. Although phospholipids are often considered simply as building blocks of membranes, an increasing number of reports demonstrates their importance and involvement in numerous other biological processes. The purpose of this review is to highlight and discuss some of these unusual, or less known, functions of phospholipids, with a special focus on PE, the most abundant glycerophospholipid class in most bacteria and the second most abundant in many eukaryotes [1]. PE, formerly called cephalin, is a zwitterionic neutral molecule adopting a conical shape due to its relatively small head group compared to its bulky hydrophobic tail. In aqueous solutions, PE shows a tendency to organize into nonlamellar structures, such as inverted hexagonal phases [8], an important property to generate lateral pressure within the bilayer to (functionally) stabilize membrane proteins and protein complexes [9,10], and to facilitate membrane fusion and fission events [11].

1.2. Trypanosoma brucei, a model organism to study eukaryotic lipid metabolism

Trypanosoma brucei is a protozoan parasite belonging to the class of Kinetoplastea [12]. It is the causative agent of Human African Trypanosomiasis, or sleeping sickness, and a corresponding animal

Abbreviations: PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; SM, sphingomyelin; IPC, inositolphosphoryl ceramide; EPC, ethanolaminephosphoryl ceramide; EPT, ethanolamine phosphotransferase; CEPT, choline/ethanolamine phosphotransferase; PSD, PS decarboxylase; NAE, *N*-acylethanolamine; GPI, glycosylphosphatidylinositol; eEF1A, eukaryotic elongation factor 1A; EPG, ethanolamine phosphoglycerol; LPS, lipopolysaccharide; DAG, diacylglycerol; AAG, alk-1-enyl-acylglycerol

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disease, called Nagana, occurring on the African continent. To complete its complex life cycle, the parasite depends on two host organisms, an insect vector (*Glossina* spp., or tsetse fly) and a mammal. As a consequence of their digenetic parasitic lifestyle, trypanosomes undergo dramatic metabolic and morphological changes to adapt to the different environments in the insect vector and the mammalian host [13,14]. Among the three subspecies of *T. brucei*, *T. brucei brucei* infects animals only, causing high economic loss in livestock farming and making it virtually impossible to breed cattle in certain regions in Africa [15], whereas *T. brucei gambiense* and *T. brucei rhodesiense* are the causative agents of the chronic and acute forms, respectively, of the human disease [16]. Human African Trypanosomiasis is recognized as a neglected tropical disease and is lethal if untreated. Unfortunately, currently available drugs show high toxicity and efficacy problems; thus, there is an urgent need for the development of new medication [17].

In addition to studying African trypanosomes to develop a cure for the diseases they cause, *T. brucei* has emerged as an interesting model organism to study eukaryote biology in general. Several important biological processes have been identified using *T. brucei* as model eukaryote, such as antigenic variation [18], trans-splicing [19], glycosylphosphatidylinositol (GPI)-anchoring of proteins [20] and RNA editing [21]. Furthermore, the genomes of several subspecies of *T. brucei* are fully sequenced and multiple comprehensive data bases on mRNA expression [22], protein expression [23] and the metabolome [24] in different life cycle forms are now available. In addition, RNA interference (RNAi) libraries have been established for functional screening of loss-of-function mutations in *T. brucei* [25,26]. Finally, trypanosomes often have single copies of organelles, such as the mitochondrion and the Golgi, making them ideal organisms to study membrane and organelle formation [27]. Recently, trypanosomatids have been placed phylogenetically at the bottom of the eukaryotic tree [28]. Experimental evidence for this repositioning includes the genetic absence of the eukaryotic origin recognition complex in several kinetoplastids [29], the functional identification of a mitochondrial outer-membrane translocase of bacterial origin [30], and the identification and characterization of a bacterial-type cardiolipin synthase in *T. brucei* [31], making trypanosomes a prime model organism to study ancient cellular processes in eukaryotes.

The phospholipid composition in *T. brucei* parasites reflects that in other eukaryotes, with PC (45–60% of total) and PE (10–20% of total) representing the major glycerophospholipid classes [32–34]. Interestingly, the relative amounts of ether-type glycerophospholipids are very high in *T. brucei* compared to many other eukaryotic cells, in particular for PE, which consists of >80% and >60% of ether-type molecular species in procyclic (insect form) and bloodstream form parasites, respectively. In addition, *T. brucei* procyclic forms contain significant levels of SM (5–10% of total) and IPC (8–12% of total), whereas bloodstream forms contain about twice as much SM and smaller amounts of EPC instead of IPC. Although *T. brucei* has long been thought to import most lipids from the environment (see below), more recently *de novo* fatty acid and phospholipid synthesis has been shown to be essential in both bloodstream and procyclic forms [35–40].

In this review, we outline and discuss how *T. brucei* can contribute, or has contributed in the past, to the current knowledge of phospholipid synthesis, in particular on PE homeostasis, in eukaryotic cells. In addition, we will point out unique features in *T. brucei* PE metabolism that may represent, or reveal, possible targets for chemotherapeutic action to combat parasitic diseases.

2. The 'ins' of PE synthesis (see Fig. 1)

2.1. Pathways for de novo synthesis of PE

De novo biosynthesis of PE can occur via three different pathways: (a) the cytidine diphosphate (CDP)-ethanolamine branch of the Kennedy pathway [41], (b) by head group exchange with PS [42], and (c) by PS decarboxylation [43]. These reactions or reaction sequences occur in a wide range of organisms; however, their relative contributions to PE formation vary considerably (reviewed by Vance and Tasseva in this Special Issue).

2.1.1. The Kennedy pathway

The pathway leading to *de novo* formation of PE and PC involving CDP-activated intermediates is referred to as the Kennedy pathway [41] (Fig. 2). The reaction sequences are active in most eukaryotic organisms, but not bacteria and archeae [44]. In the CDP-ethanolamine branch, ethanolamine is phosphorylated by ethanolamine kinase to ethanolamine phosphate, which becomes activated to CDP-ethanolamine by ethanolamine-phosphate cytidylyltransferase. Both reactions occur in the cytosol, with the second enyzme catalyzing the rate-limiting step in PE synthesis [45]. The last step in PE formation is mediated by two different enzymes: choline/ethanolamine phosphotransferase (CEPT) catalyzes transfer of CDP-ethanolamine to diacylglycerol (DAG), a reaction shared by the ethanolamine and choline branches of the Kennedy pathway [46]. In contrast, ethanolamine phosphotransferase (EPT) is specific for the ethanolamine branch and mediates attachment of CDP-ethanolamine to ether-type glycerol moieties, alkyl-acylglycerols or alk-1-enyl-acylglycerols (AAGs), as demonstrated in mammalian cells [47,48]. Both enzymes are membrane-bound, with CEPT localizing to the ER and nuclear membrane in mammalian cells [49], while the localization of EPT is unknown.

In *T. brucei*, PE synthesis via the CDP-ethanolamine pathway (Fig. 2) is essential for parasite growth, validating the Kennedy pathway as potential drug target. Down-regulation by RNAi of one of ethanolamine kinase, ethanolamine-phosphate cytidylyltransferase, CEPT, or EPT results in growth arrest of *T. brucei* procyclic forms in culture [35]. The specific depletion of diacyl-type and ether-type PE molecular species in parasites after down-regulation of CEPT and EPT, respectively, demonstrates the selectivity of the two enzymes towards the different glycerol-based substrates [35]. In addition, the study shows that the two pools of PE cannot substitute for each other, i.e. in the absence of ether-type PE synthesis, diacyl-type PE is unable to compensate for its functions, demonstrating that both subclasses of PE have distinct (and essential) functions in membranes [50]. At present, it is unclear if the observed selectivity of CEPT and EPT in *T. brucei* is due to the localization of the enzymes and their respective substrates in distinct organelles, or if formation of the different PE subclasses is determined by the substrate specificity of CEPT and EPT for DAG and AAG, respectively, as has been shown in yeast and mammalian cells [48]. Ether-lipid metabolism in *T. brucei* is associated with glycosomes [51], specialized peroxisome-like organelles containing glycolytic enzymes that are present in all members of the Kinetoplastea [52]. Thus, it can be speculated that the final step in formation of ether-type PE molecular species, mediated by EPT, may also localize to the glycosomes, i.e. the site of the location of the enzymes catalyzing AAG formation [53–55]. We are currently addressing this question in *T. brucei* procyclic forms by expressing epitope-tagged constructs of EPT and CEPT. Furthermore, it has been reported that down-regulation of PE synthesis severely affects *T. brucei* mitochondrial morphology and function [56], highlighting the importance of PE in mitochondrial homeostasis.

2.1.2. Decarboxylation of PS

In many organisms, decarboxylation of PS is the major pathway for PE formation [57]. PS decarboxylases (PSDs) can be divided into one class comprising all bacterial enzymes and two classes comprising the eukaryotic isoforms [58]. In yeast, the PSD1 isoform located in mitochondria is responsible for production of bulk cellular PE, whereas PSD2 located in the Golgi produces mainly PE for methylation to PC [59]. Mammalian cells only contain the mitochondrial PSD1 isoform. Knocking-out PSD1 in mice results in severe mitochondrial defects and embryonic lethality [60]. In *Arabidopsis*, lack of PSD activity does not affect growth, but results in depletion of the mitochondrial PE