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FUNCTIONAL ROLES OF LIPID METABOLITE CDP DIACYLGLYCEROL AND ITS SYNTHASE ENZYMES: RECENT DEVELOPMENTS

Manoj G Tyagi[1], Dharmendra Singh[1], Nirmala Joyce[2], Mohammed Amil[1] and Shyam S Yadav[1]

[1]NIMS College of Paramedical Technology, NIMS University, Jaipur, Rajasthan

[2]Department of Chemical Engineering, IIT Chennai, Tamilnadu

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Address for Correspondence: Manoj G Tyagi, NIMS College of Paramedical Technology, NIMS University, Jaipur, Rajasthan, India
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ABSTRACT:
Phospholipids are basic building-block molecules for biological membranes. Biosynthesis of phospholipids i.e phosphatidylinositol, phosphatidylglycerol and phosphatidylserine requires a central liponucleotide intermediate named cytidine-diphosphate diacylglycerol (CDP-DAG). The CDP-DAG synthase (CDS) is an integral membrane enzyme catalysing the formation of CDP-DAG, an essential step for phosphoinositide recycling during signal transduction. New roles are being ascribed to the CDP-DAG in signalling and pathophysiological conditions. This pathway may also be the target of novel drugs to be used in neuro-psychiatric conditions.

Keywords: Phospholipids, Cytidine diphosphate, diacylglycerol, phosphatidic acid, phosphatidyl-glycerol.

INTRODUCTION

Glycerophosphate-based phospholipids are the important components of biological membranes, including the plasma membrane and organelle membranes. Several different glycerophospholipid species with diverse head groups exist in various biological membranes, including phosphatidylglycerol (PG), cardiolipin, phosphatidylcholine, phosphatidyl-ethanolamine, phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidic acid (PA) [1]. Cytidine intermediates play a key role in the biosynthesis of all classes of glycerol phospholipids, and the enzymes catalyzing their formation are thought to catalyze the rate-controlling steps in their respective pathways [2]. The biosynthetic pathways to phosphatidylinositol, phosphatidylcholine, and phosphatidylethanolamine are all 2-component systems composed of acytidylyl transferase followed by a synthase. In the 1960s, Kennedy and co-workers [3] discovered that a liponucleotide, named cytidine-diphosphate diacylglycerol nowknown as CDP-DAG, is a key precursor for the biosynthesis of PI9, PG10,11 and PS12. These previous works also demonstrated that CDP-DAG is at the branch point of diverse biochemical pathways leading to the synthesis of phospholipids with various head groups [4]. The CDP-DAG-mediated pathways are pivotal sources for the

de novo synthesis of phospholipids in both prokaryotes and eukaryotes [5]. CTP:phosphocholine cytidylyltransferase is the most studied enzyme and is the rate-controlling step in phosphatidylcholine biosynthesis. Accordingly, transient overexpression of this cytidylyltransferase in COS cells yielded a 3 to 5 fold increase in the incorporation of $^{[3H]}$choline into phosphatidyl-choline and a significant elevation in cellular CDP-choline [6]. In mammals, there are two CDS enzymes and they are CDS1 and CDS2 (1). CDS enzymes catalyze the formation of CDP-DAG from PA, the precursor for all phospholipids and TAG synthesis (Refer Fig.1).CDS1 and CDS2 are believed to localize to the ER, where they regulate the synthesis of phosphatidylinositol (PI) and phosphatidylglycerol (PG) [7,8]. Although the biochemical functions of CDS1 and CDS2 have been characterized, still less is known about their involvement in cellular lipid storage and adipocyte differentiation. This review article outlines the recent developments in this area of lipid biochemistry and projects on the possible biological functions and its target of the CDP-DAG synthase for clinical therapeutics.

Cytidine diphosphate DAG synthase (CDS) enzymes: As of now only two CDS isoforms in mammals have been identified and characterized (Figure 2). Both of these enzyme isoforms are believed to be localized to the endoplasmic reticulum (ER).In mammals, two homologous genes of CDS (CDS1 and CDS2) have been cloned that are 73% identical and 92% similar. Human CDS1 is 461 amino acids long and has a calculated molecular weight of ~53 kDa whilst CDS2 is 444 amino acids with a calculated molecular weight of ~51 kDa. It was believed that CDS1 was present in mitochondria for synthesizing cardiolipin [9]. However, a recent study using yeast has shown that the enzyme Tam 41 is responsible for this activityand that CDS1 does not reside in the mitochondria, although its presence may affect mitochondrial lipid composition [10]. CDS1 and CDS2 are expressed in a variety of tissues. In mice, CDS1 is found in adult brain, eye, smooth muscle, and testis [11,12]. In the eyes, CDS1 is strongly expressed in the photoreceptor layer of adult retinas, which could suggest a role for CDS1 in phototransduction.This is suggestive of its possible role in retinopathy particularly the diabetic retinopathy. It was believed that CDS1 was present in mitochondria for synthesizing cardiolipin. However, a recent study using yeast has shown that the enzyme Tam 41 is responsible for this activityand that CDS1 does not reside in the mitochondria, although its presence may affect mitochondrial lipid composition.CDS1 and CDS2 are expressed in a variety of tissues. CDS2 has a broad expression pattern and was found in virtually every tissue, however, some discrepancies exist in the tissue localization of CDS2. For example, another study showed that an arachidonoyl-preferring CDS i.e CDS2 based on recent results was expressed only in the brain, eye, and testis. The roles of CDS1 and CDS2 have primarily been studied inphosphatidylinositol (PI) synthesis. Many of the cellular functions attributed to CDS enzymes are believed to result from their role in generating the precursor for phosphatidylinositol 4,5-bisphosphate (PIP_2), a potent signaling precursor molecule. For example, phototransduction signaling in vertebrate and invertebrate systems is believed toproceed, at least partly, via phosphoinositide signalling. Thus its role in the pathophysiology of the eye seems pivotal and requires further investigation.

***De novo* synthesis of DAG :**

1,2 diacylglycerol is a glycerophospholipid metabolite downstream of the phospholipase C pathway. There are two main pathways of DAG synthesis in yeast and mammals [13] in one, DAG is synthesized from glycerol-3-phosphate (as a result of triacylglycerol mobilization);in the other, it is generated from dihydroxyacetone-3-phosphate (a glycolysis intermediate). These two precursors undergo

several modifications including two acylation steps that give rise first to lysophosphatidic acid. (LPA) and then to phosphatidic acid (PA); the later is subsequently transformed into DAG through the action of PA phosphohydrolases.

Measurement of CDP-diacylglycerol in cultured cells:

PC12 cells obtained from ATCC (VA, USA) and were maintained in RPMI-1640 Medium supplemented with 5% fetal bovine serum, 10% horse serum and 2 mM L glutamine at 37°C with 5% CO2 aeration. The cells were cultured on poly-D-lysine coated plates until reaching approximately 80% confluency and then transferred to Neurobasal medium i.e Neurobasal+N2 supplement +glutamine one day before the assay. Cells in wells of a 24- well plate were labeled with 1.5 µCi [5-3H]cytidine (20 Ci/mmol; ARC, St. Louis, MO) for 30 min to generate a pool of radiolabeled cytidine triphosphate (CTP). After addition of 5 mM LiCl, solutions of test drugs were added to the cells at indicated concentrations and incubation continued for duration of 3 h. To terminate this reaction, 1.5 ml of chloroform-methanol-1 M HCl (100:200:1) was added with mixing. The lipids were extracted by partitioning to the chloroform layer as previously described ; aliquots were than quantitatively transferred to polypropylene tubes and were dried overnight at room temperature. Biosafe scintillation cocktail was added to each sample and the radioactivity determined by liquid scintillation. The radioactivity in each sample relates to [^{3}H]CDP-diacylglycerol as characterized by previous studies [14].

Hydrophilic interaction liquid chromatography (HILIC) and fractionation of lipid metabolite like the CDP diacylglycerol:

This technique also known as, HILIC is used for the fractionation of total lipid extracts into lipid classes using the Spherisorb Si column (250 ×4.6 mm, 5 mm, Waters), a flow rate of 1 mL/min, an injection volume of 10 mL, separation temperature of 40 ◦C and a mobile phase gradient: 0 min – 94% A + 6% B, 60 min – 77% A + 23% B, where A is acetonitrile and B is 5 mM aqueous ammonium acetate. The injector needle is washed with the mobile phase after each injection.

Lipid classes are identified using ESIMS in the mass range *m/z* 50–1500 with the following setting of tuning parameters: pressure of the nebulizing gas of 60 psi, the drying gas flow rate of10 L/min and temperature of the drying gas 365 ◦C. Fractions of lipid classes are collected manually, evaporated by a mild stream of nitrogen and redissolved in the initial mobile phase composition for the 2D analysis. The volume for redissolvation is selected according to the concentration of individual fractions in the range of 0.1 to 1 Liter as described earlier [15].

Pathophysiological implications for CDP diacylglycerol:

The surplus levels of free fatty acids contributes to liver failure in obesity and in type 2 diabetes [16,17]. Although fatty-acid-induced liver disease is generally attributed to triglyceride accumulation in liver cells, recent data indicate that triglyceride accumulation might also have a protective role. The high toxicity of saturated fatty acids is partly due to a limited capacity of the liver cells to incorporate them into triglycerides [18]. The acyl chain selectivity of CDS2 is similar to that of DGKε,which was shown to be required for the arachidonoyl enrichment of PI species. CDS2 could play a similar yet greater role in the enrichment of PI with an arachidonoyl chain. CDP-DAG produced by CDS2 can be used only for the synthesis of phospholipids. Conversely, PA synthesized by DGKε can be used for signal transduction pathways and structurally, for phospholipid synthesis and can be dephosphorylated back to diacylglycerol by the enzyme phosphatidic acid phosphatase. Furthermore, expression studies indicatethat CDS1 and CDS2 exhibit quite different tissue

specificity. In the mouse, CDS2 appears to be ubiquitously expressed whilst CDS1 has a restricted pattern of expression [19]. CDS1 has been shown to be highly expressed in the heart and in SHHF i.e spontaneously hypertensive heart failure rats, an increase in CDS1 mRNA was observed with increasing age whilst CDS2 mRNA decreased during heart failure development [20]. The increase in CDS1 mRNA corresponded to an increase in mitochondrial CDS activity with no change in microsomal CDS activity. It is quite likely that CDP diacylglycerol pathway may be modulating the synthesis of prostaglandins for e.g the prostacyclin and also PI-4 kinase levels [21,22]. Recent data also demonstrates that various anti-depressants increase phosphatidylinositol and CDP-diacylglycerol synthesis probably through stimulation of the enzymatic activity of CDS, the enzyme that synthesizes CDP-diacylglycerol. While not all antidepressant agents depend on serotonin signaling for their actions [23].

CDP diacylglycerol pathway in microbes: Prokaryotes lack the enzyme to produce DAG, and deploy CDP-DAG as the progenitor for all glycerophospholipids. The enzyme CDP-DAG synthase (CDS) is therefore regarded as one of the most central enzymes of lipid synthesis in prokaryotes as well as in eukaryotes. Given the functional integration of apicoplast with other organelles harboring lipid synthesis, understanding the mechanisms and physiological importance of CDP-DAG synthesis is particularly interesting in the prokaryotic cells. The localization of mycobacterial CDS activity in membranes was not surprising, since these enzymes are known to be membrane-bound and usually require non-ionic detergents for their solubilization and puri®cation. So far, the partial puri®cation of CDS enzymes has only been achieved to near homogeneity for *E. coli* [24]. The deduction of the *M. tuberculosis* genome [25] revealed an open reading frame (Rv2881c, *cdsA*), which is homologous with *E. coli* CDS. The predicted gene product is expected to have a molecular mass of 32 kDa and to contain eight putative transmembrane domains [25]. Prokaryotes lack the enzyme to produce DAG, and deploy CDP-DAG as the progenitor for all glycerophospholipids [26]. The enzyme CDP-DAG synthase (CDS) is therefore regarded as one of the most central enzymes of lipid synthesis in prokaryotes as well as in eukaryotes [27]. Given the functional integration of apicoplast with other organelles harboring lipid synthesis, understanding the mechanisms and physiological importance of CDP-DAG synthesis is particularly interesting in the prokaryotic cells.

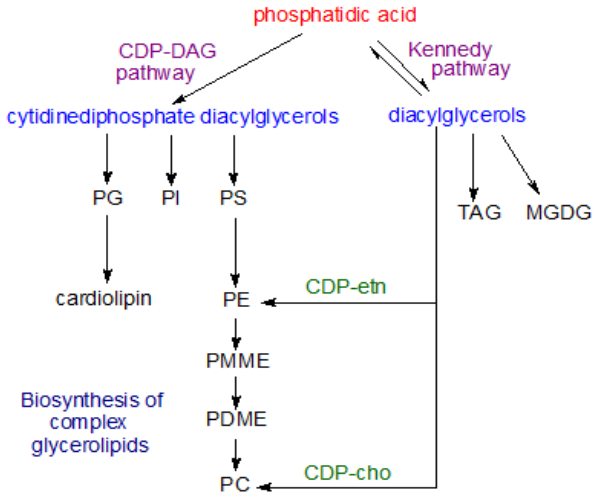
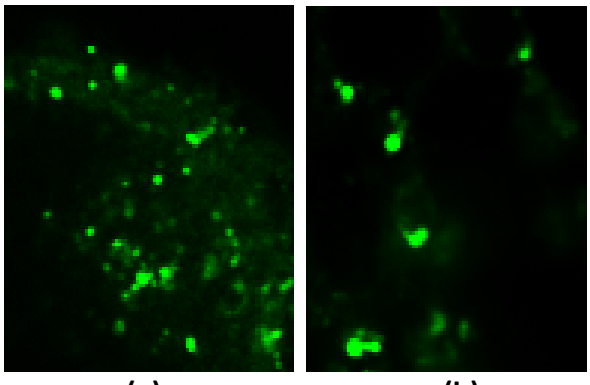


Figure 1: Synthesis of CDP diacylglycerol and downstream phospholipids



(a) **(b)**

Figure 2: CDS1 (a) and CDS2 (b) enzymes as seen in the HeLA cells transfected using Green fluoroscent proteins.

Conclusion:

The lipid composition of cellular organelles is tailored to suit their specialized functions. A fundamental transition in the lipid landscape divides the secretory pathway in early and late membrane territories, allowing an adaptation from biogenic to barrier functions. Recent studies suggest that Lipid synthase enzymes like the CDS1 and CDS1 may be contributing in pathophysiological conditions and in the signaling mechanisms mediated by hormones and neurotransmitters as well as drugs. Considering the fact that phosphatidic acid is the precursor for the synthesis of CDP diacylglycerol the phospholipase D and its interaction with this pathway needs more research investigation. More research studies are required to probe the functional roles of these enzymes in eukaryotic and prokaryotic cells. Novel drugs affecting these enzymes could be adjuvants in clinical therapeutics.

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