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The *Arabidopsis thaliana* lysophospholipid acyltransferase At1g78690p acylates lysocardiolipins

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ABSTRACT

The *Arabidopsis thaliana* lysophospholipid acyltransferase At1g78690 acylates a variety of lysophospholipids such as lyso phosphatidylglycerol, lyso phosphatidylethanolamine and lyso phosphatidylserine. Despite di-acylate phosphatidylglycerol being a substrate, overexpression of At1g78690 in *Escherichia coli* leads to the accumulation of acyl-PG. Here we show that cardiolipin also accumulates in cells overexpressing At1g78690. To help try and explain this observation, we show, using a liquid chromatography mass spectrometry (LC-MS) based assay, that At1g78690 utilizes both mono- and di-lyso cardiolipin as an acyl acceptor. Because At1g78690 shares high homology (~40%) with the cardiolipin remodeling enzyme tafazzin, we also tested whether At1g78690 was able to catalyze a tafazzin-like transacylation reaction. Di-linoleoyl phosphatidylcholine was used as the acyl donor and mono-lyso cardiolipin was used as the acyl acceptor in a reaction and the reaction was monitored by LC-MS. No transfer of the linoleoyl chains was detected in an At1g78690 dependent manner suggesting that, despite the strong homology, these enzymes catalyze unique reactions.

1. Introduction

The *Arabidopsis thaliana* gene At1g78690 encodes a lysophospholipid acyltransferase that utilizes acyl-CoA to acylate mono-acylated glycerophospholipids (GPLs) to their di-acylated GPLs *in vitro* [1,2] yet when it is overexpressed in *Escherichia coli* the headgroup acylated GPL acyl phosphatidylglycerol (acyl PG) accumulates. At1g78690 does not, however, acylate the headgroup of PG directly [1]. Recently we have shown that At1g78690 also acylates *bis*(monoacylglycero)phosphate (BMP) to form acyl-PG [2]. This activity potentially explains the accumulation of acyl PG in *E. coli* when At1g78690 is overexpressed *in vivo* – endogenous BMP found in *E. coli* is acylated to acyl PG *in vivo* leading to increased levels of acyl PG.

At1g78690's closest protein homolog is the transacylase tafazzin [3]. Tafazzin has been implicated in remodeling cardiolipin, a tetra-acylated GPL [4,5]. A mutation in the gene encoding for tafazzin, leads to an X-linked cardiomyopathy known as Barth's syndrome [6–8]. Cells deficient in tafazzin have a decreased levels of cardiolipin (CL) and elevated levels of monolysocardiolipin (MLCL), allowing MLCL/CL ratios to serve as a diagnostic tool for Barth's syndrome [4].

As related above, tafazzin is involved in the remodeling of the cardiolipin acyl chains, converting *de novo* synthesized CL to primarily tetralinoleoyl CL [9]. Tafazzin catalyzes the acylation of lyso phosphatidylcholine (lyso PC) from acyl chains derived from tetra-acylated CL, and also catalyzes the reverse reaction, acylating MLCL from acyl chains derived from PC. Tafazzin displays broad acyl chain specificity under certain *in vitro* conditions [5,10], however, promotes specific remodeling of CL to tetralinoleoyl CL in the context of non-bilayer lipid membranes [11].

At1g78690 shares 39% and 41% sequence homology to human and *Drosophila melanogaster* tafazzins, respectively, indicative of a shared fold and enzymatic mechanism. Indeed, At1g78690 is likely the *A. thaliana* version of tafazzin and suggests that At1g78690 may possess tafazzin-like activity or play a role in modulating CL composition *in vivo*.

In eukaryotes, CL is found in the inner mitochondrial membrane in eukaryotes and is critical for mitochondrial function [12]. It serves as a necessary proton trap for oxidative phosphorylation and is a trigger for apoptosis. In bacteria such as *E. coli*, CL is also enriched at the cell poles and cell division sites [13,14] and is involved in the osmotic stress response [15].

Here we show that expression of At1g78690 impacts the levels

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Abbreviations

LC-MS	liquid chromatography mass spectrometry
GPL	glycerophospholipid
acyl PG	acyl phosphatidylglycerol
PC	phosphatidylcholine
PS	phosphatidylserine
PI	phosphatidylinositol
PE	phosphatidylethanolamine
BMP	bis(monoacylglycerol)phosphate
CL	cardiolipin
MLCL	monolyso cardiolipin
DLCL	dilyso cardiolipin
LPLAT	lysophospholipid acyltransferases
AGPAT	1-acyl-glycerol-3-phosphate O-acyltransferase (AGPAT)

of CL in *E. coli* in addition to the acyl-PG levels. In addition, because of the high homology of At1g78690 to tafazzin we investigated the enzyme's ability to acylate MLCL and DLCL as well as whether At1g78690 possesses taffazin-like MLCL:PC transacylase activity.

2. Materials and methods

2.1. Materials

Solvents for lipid extraction were reagent grade from Sigma. High performance liquid chromatography solvents were CHROMASOLV® Plus, HPLC grade from Sigma Aldrich. Other chemicals were purchased from VWR or Sigma–Aldrich.

Heart bovine monolysocardiolipin (MLCL), dilysocardiolipin (DLCL), 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine (18:2 PC), and 20:5-Coenzyme A were from Avanti Polar Lipids (Alabaster, AL).

2.2. Growth of E. coli and preparation of protein extracts

E. coli BLR(DE3)pLysS were transformed with pET15b and pHis-AT1g78690Lipid and grown as described previously [1]. Following harvesting by centrifugation for 20 min at 2600 x g, cell pellets were washed with 15 mM Tris, pH 7.4 and the centrifugation repeated to harvest cells. Cell pellets were frozen at −80 °C until further use.

Cell-free extracts and membranes were prepared as described previously [2]. Protein concentrations were determined using a bicinchoninic acid reagent (Thermo Scientific) with bovine serum albumin as the standard. All protein samples were stored at −80 °C until needed.

2.3. Preparation of in vitro enzyme products

In vitro products were generated in a 0.5 ml reaction that contained 100 μM 20:4 or 20:5-CoA, 300 μM acyl acceptor (MLCL, DLCL, or 1-acyl lyso GPL), 15 mM Tris pH 7.4, and 0.05% Triton X-100, and were initiated by the addition of 0.5 mg/mL membranes from BLR(DE3)pLysS/pET15b or BLR(DE3)pLysS/pHis-At1g78690 prepared as described previously [2]. For transacylation assays, 100 μM di-linoleoyl PC was included in place of the acyl CoA, and 300 μM MLCL as the acyl acceptor. Reactions were incubated at 37 °C for 3 h and terminated by Bligh-Dyer extraction [2,16]. The lower phase of the two-phase system was transferred to a glass vial equipped with a glass volume reducer, dried under a stream of N_2 gas and stored at −20 °C until analysis.

2.4. Mass spectrometry of in vitro products

The dried products from each *in vitro* acylation or transacylation reaction was re-suspended in 100 μL $CHCl_3$:CH_3OH (2:1 v/v) and analyzed using normal phase liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometer as described previously [2].

3. Results

3.1. Overexpression of At1g78690 leads to the accumulation of CL in addition to acyl PG

Previously we have shown that overexpression of At1g78690 leads to the accumulation of the headgroup acylated GPL acyl PG [1]. Further examination of the LC-MS profile reveals that, in addition, a number of cardiolipin species accumulate. Fig. 1 shows that the total amount of cardiolipin increases in lipid extracts derived from BLR(DE3)pLysS cells expressing At1g78690 compared to lipid extracts prepared from cells expressing pET vector alone. Based on the *m/z* of the CLs, the CLs range from CL with 62:1 (total carbons:total unsaturations in the acyl chains) at *m/z* 1321.919 to 70:3 at *m/z* 1430.003.

3.2. MLCL and DLCL acylation

At1g78690 robustly acylates 1-acyl lyso phosphatidylethanolamine (PE), 1-acyl lyso PG, 1-acyl lyso phosphatidylserine (PS), and 1-acyl phosphatidylinositol (PI) using acyl-CoA as the acyl donor to produce PE, PG, PS, and PI, respectively [1,2]. Since heterologous expression of At1g78690 in *E. coli* impacts the lipid profile by altering CL levels, and because At1g78690 has been shown to acylate various lysophospholipids, we hypothesized that At1g78690 may be acylating endogenous under-acylated CLs to form CL. Using a LC-MS based assay, we assessed whether At1g78690 can catalyze the acylation of DLCL to form MLCL, and if At1g78690 can acylate MLCL to CL. Using 20:5 acyl-CoA as the acyl donor and MLCL or DLCL as the acyl acceptor we monitored the formation of the expected products using membranes prepared from cells expressing At1g78690, and compare these products to those formed from the reaction using membranes expressing vector alone. For both acyl acceptors, MLCL and DLCL, both of which are predominantly acylated with linoleate (18:2), the expected acylated products CL and MLCL were detected (Fig. 2). In Panel A, the $[M\text{-}H^+]^-$ at *m/z* 1469.972 corresponds to the expected CL product with three 18:2 chains and a fourth 20:5 chain added by At1g78690. In Panel B, the $[M\text{-}2H^+]^{2-}$ at *m/z* 603.365 corresponds to the expected MLCL product with two 18:2 chains and a third 20:5 chain added by At1g78690.

3.3. MLCL:PC transacylation

Tafazzin has been shown to have phospholipid transacylase activity, capable of transferring the acyl chain from PC to acylate MLCL, forming CL and lyso PC [3–5]. Using a similar LC-MS based assay, we tested for taffazin-like transacylase activity in membranes with At1g78690 as the enzyme source. Using MLCL as the acyl acceptor and 18:2 PC as the acyl donor, we monitored the incorporation of linoleoylate into CL and other GPLs. No CLs with *m/z*'s consistent with 18:2 acyl chains incorporation were detected in an At1g78690-or PC-dependent manner. Fig. 3 shows the region of the negative ion MS where the expected CL ion (*m/z* 1447.9649) would be present. There was no detectable difference in the levels