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Characterisation of acyltransferases from *Synechocystis* sp. PCC6803 ☆

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Abstract

As phylogenetic ancestors of plant chloroplasts cyanobacteria resemble plastids with respect to lipid and fatty acid composition. These membrane lipids show the typical prokaryotic fatty acid pattern in which the *sn*-2 position is exclusively esterified by C_{16} acyl groups. In the course of de novo glycerolipid biosynthesis this prokaryotic fatty acid pattern is established by the sequential acylation of glycerol-3-phosphate with acyl-ACPs by the activity of different acyltransferases. In silico approaches allowed the identification of putative *Synechocystis* acyltransferases involved in glycerolipid metabolism. Functional expression studies in *Escherichia coli* showed that *sll1848* codes for a lysophosphatidic acid acyltransferase with a high specificity for 16:0-ACP, whereas *slr2060* encodes a lysophospholipid acyltransferase, with a broad acyl-ACP specificity but a strong preference for lysophosphatidylglycerol especially its *sn*-2 acyl isomer as acyl-acceptor. The generation and analysis of the corresponding *Synechocystis* knockout mutants revealed that lysophosphatidic acid acyltransferase unlike the lysophospholipid acyltransferase is essential for the vital functions of the cells.

Keywords: *Synechocystis*; Phospholipid; Lysophospholipid; Lysophosphatidic acid; Lysophosphatidyl-glycerol; Lysophosphatidic acid acyltransferase; Lysophosphatidylglycerol acyltransferase; Acyl-ACP; Acyl-CoA; Knockout mutant

As phylogenetic ancestors of plant chloroplasts cyanobacteria resemble plastids with respect to lipid and fatty acid composition [1,2]. They possess the two neutral galactolipids monogalactosyldiacylglycerol (MGD) and digalactosyldiacylglycerol (DGD) as major components and the two anionic glycerolipids sulfoquinovosyldiacylglycerol (SQD) and phosphatidylglycerol (PG) as minor components (Fig. 1). These membrane lipids as well as those synthesised in plastids show the typical prokaryotic fatty acid pattern in which the *sn*-2 position is exclusively esterified by C_{16} acyl groups while the *sn*-1 position predominantly carries C_{18} acyl groups (Fig. 1).

In the course of de novo glycerolipid biosynthesis this prokaryotic fatty acid pattern is established by the sequential acylation of glycerol-3-phosphate (G3P) with acyl-ACP to 1-acylglycerol-3-phosphate (lysophosphatidic acid, LPA) and 1,2-diacylglycerol-3-phosphate (phosphatidic acid, PA) catalysed by a glycerol-3-phosphate (GPAT) and a lysophosphatidic acid acyltransferase (LPAAT). The analysis of the fatty acid specificities and selectivities of the plastidial GPATs and LPAATs from different plants showed that the GPATs possess a more or less pronounced selectivity for the 18:1 acyl group while the LPAATs exclusively direct 16:0 to the *sn*-2 position, so that the prokaryotic fatty acid pattern

☆ *Abbreviations:* G3P, glycerol-3-phosphate; LPA, lysophosphatidic acid; LPG, lysophosphatidylglycerol; LPE, lysophosphatidylethanolamine; LPAAT, lysophosphatidic acid acyltransferase; LPLAT, lysophospholipid acyltransferase; GPAT, glycerol-3-phosphate acyltransferase; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; MDG, monogalactosyldiacylglycerol; DGD, digalactosyldiacylglycerol; SQD, sulfoquinovosyl diacylglycerol.

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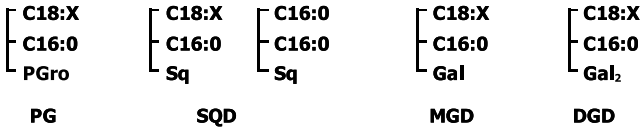

Fig. 1. Predominant glycerolipid species from *Synechocystis* sp. PCC 6803 showing the typical prokaryotic fatty acid pattern (PG, phosphatidylglycerol; MGD, monogalactosyldiacylglycerol; DGD, digalactosyldiacylglycerol; SQD, sulfoquinovosyldiacylglycerol; PGro, phosphoglycerol; Sq, sulfoquinovose, Gal, galactose; C18:X, stearoyl, oleoyl, linolyl, and linolenoyl acyl groups, C16:0, palmitoyl group).

is established [3]. In vivo labelling experiments suggest that the cyanobacterial GPATs and LPAATs determine the prokaryotic fatty acid pattern by directing C18:0 to the *sn*-1 and C16:0 to the *sn*-2 position in a similar way as the plastidial enzymes [4]. Unlike the plastidial acyltransferases, which have been characterised and the respective genes of which have been cloned [5–9], information regarding cyanobacterial acyltransferases is limited to reports concerning acyl-ACP specific lysoglycerolipid acyltransferases of *Anabaena variabilis* [10,11] and in vivo feeding experiments with *Synechocystis* sp. PCC6803 [4].

The known genome sequence of *Synechocystis* allows in silico approaches to identify four putative acyltransferases (SLL1848; SLL1737, SLR2103, and SLR2060) containing the different conserved acyltransferase motifs jointly responsible for the catalytic activity [5,12] and thereby providing new opportunities to characterise the different acyltransferases and to investigate the importance of this enzymic activities involved in glycerolipid metabolism.

In this paper, we report on the functional characterisation of the *Synechocystis* genes *sll1848* and *slr2060* and show the in vivo function of the encoded acyltransferases by generating and analysing the corresponding knockout *Synechocystis* mutants.

Materials and methods

Bacterial strains and growth conditions. *Synechocystis* sp. PCC6803 cultures were obtained from the Pasteur Culture Collection and maintained according to Castenholz [13]. *Escherichia coli* (*E. coli*) strains XLI blue and DH5α were used for cloning and propagation of recombinant plasmids [14]. The *E. coli* mutants BB2636 [15] and JC201 [16] were used for heterologous complementation experiments and for functional expression studies of recombinant *Synechocystis* acyltransferases.

Development of pQE1848 and pQE2060 expression vectors. Genomic DNA from *Synechocystis* cells was extracted according to Rippka [17]. The open reading frames corresponding to the *sll1848* and *slr2060* genes from *Synechocystis* were amplified by PCR with *Pfu* polymerase (Stratagene, LaJolla, CA, USA) using the specific primer pairs 1848-F: 5′-GGATCCGTGGATTCCGAGATTAATC-3′ and 1848-R: 5′-GAGATCTTTAATCCCTGCCTAAATCC-3′ and 1848His-R: 5′-GAGATCTATCCCTGCCTAAATCC-3′ containing *Bam*HI and *Bgl*II sites as well as 2060-F: 5′-TACCATGGAATCCCCCATCCAAGC-3′ and 2060-R: 5′-CAGATCTTTAAATAATTAGTGCGGATAACTT-3′ or 2060His-R: 5′-TCAGATCTAATAATTAGTGCGGATA-3′ containing *Nco*I and *Bgl*II sites, respectively. The amplified DNA fragments were ligated into the corresponding *Bam*HI/*Bgl*II site of the pQE60 vector (Qiagen, Hilden, Germany), creating pQE1848, and pQE1848+ and the pQE70 vector, yielding pQE2060 and pQE2060+, so that in case of pQE1848+ and pQE2060+ the 6× His-tag sequence of the respective vector was added in frame to the 3′ end of the open reading frame. As positive control for complementation studies with JC201 cells and Western blot analysis of isolated membrane fractions, the open reading frame of the *pls*C gene encoding the LPAAT from *E. coli* [18], was amplified from genomic DNA [14] by PCR with *Pfu* polymerase (Stratagene, La Jolla, USA) using the specific primer pairs plsC-F: 5′-CCATGGTATATATCTTTCGTCTT-3′ and plsC-R: 5′-GAGATCTAACTTTTCCGGCGGCT-3′ containing *Nco*I and *Bgl*II sites, respectively. The amplified DNA fragment was ligated into the corresponding *Nco*I/*Bgl*II site of the pQE60 vector (Qiagen, Hilden, Germany), creating pQEplsC+.

Heterologous complementation of BB2636 and JC201 cells. BB2636 cells [15] harbouring the repressor plasmid pREP4 and one of the expression constructs or the empty vector as control were plated on M56LP-agar plates containing 50 μg ml^{-1} carbenicillin, 25 μg ml^{-1} kanamycin, 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and 0.1% glycerol [15]. As a control cells were grown in the presence or absence of glycerol and IPTG, respectively. JC201 cells [16] harbouring the repressor plasmid pREP4 and one of the respective expression constructs or the empty vector as a control were plated on LB-agar plates containing 50 μg ml^{-1} carbenicillin and 25 μg ml^{-1} kanamycin with or without 1 mM IPTG and grown at 30, 37 or 42 °C [16,17].

Functional expression in E. coli. JC201 cells harbouring the repressor plasmid pREP4 were transformed either with the empty vector pQE60 or with one of the expression constructs grown at 30 °C in LB medium supplemented with 50 μg ml^{-1} carbenicillin and 25 μg ml^{-1} kanamycin and harvested 2 h after induction with 1 mM IPTG. LPAAT activity in subcellular fractions prepared from transgenic JC201 cells was measured in a similar way as described previously [19]. Briefly, the standard assay contained in a 50 μl reaction volume 100 mM Tricine–NaOH, pH 8.2, 12 μM [1-^{14}C]acyl-ACP (130 dpm/pmol), 40 μM *sn*-1-oleoylglycerol-3-phosphate, and enzyme sample (up to 5 μg protein). The reaction products were extracted and separated by TLC in chloroform:pyridine:formic acid (50:30:7). LPLAT activities were determined in a similar way as LPAAT activities, but the assays consisted of CHES–NaOH buffer, pH 9, 5 μM [1-^{14}C]acyl-ACP and 50 μM of 1-oleoylglycerol-3-phosphate. Acyl-ACP synthase and [1-^{14}C]acyl-ACP thioesters were prepared according to Shanklin [20] except, that [1-^{14}C]acyl-ACP was finally purified over a Sephadex-G25 column. 1-Acyl and 2-acyl lysolipids were prepared by incubating the respective glycerolipid with *Rhizopus arrhizus* lipase (Sigma–Aldrich, München, Germany) or Phospholipase A_2 (Sigma–Aldrich) and separated and purified by TLC. Protein concentrations were estimated by Bradford protein assays using bovine serum albumin as standard. Standard methods were used for SDS–PAGE analysis [21]. For Western blot analysis, proteins were transferred to PVDF membranes by semi-dry blotting and the His-tagged recombinant proteins were detected by chemiluminescence (LAS-1000, Raytest, Straubenhardt, Germany) using penta-His antibodies from mice (Qiagen, Hilden, Germany) and goat anti-mouse IgG-POD conjugate antibodies (Qiagen, Hilden, Germany) with Lumi-Light plus (Roche, Mannheim, Germany) as substrate. Pre-stained and His-tagged (Best Of All) protein markers were obtained from New England Biolabs (Beverly, MA, USA) and Biomol (Plymouth Meeting, PA, USA), respectively.

Generating Synechocystis sll1848::Cm^R and slr2060::Cm^R mutants. The open reading frames (ORF) *sll1848* and *slr2060* were amplified from *Synechocystis* genomic DNA and used to create *Synechocystis*