Biochemical and Biophysical Research Communications xxx (2017) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Disturbance of cell-size determination by forced overproduction of sulfoquinovosyl diacylglycerol in the cyanobacterium *Synechococcus elongatus* PCC 7942

Norihiro Sato [a, *], Yuki Ebiya [a], Ryutaro Kobayashi [a], Yoshitaka Nishiyama [b], Mikio Tsuzuki [a]

[a] School of Life Sciences, Tokyo University of Pharmacy and Life Sciences, Horinouchi, Hachioji, Tokyo 192-0392, Japan
[b] Department of Biochemistry and Molecular Biology, Graduate School of Science and Engineering, Saitama University, 255 Shimo-Okubo, Sakura-ku, Saitama 338-8570, Japan

ARTICLE INFO

Article history:
Received 17 April 2017
Accepted 23 April 2017
Available online xxx

ABSTRACT

Sulfoquinovosyl diacylglycerol (SQDG) is present in the membranes of cyanobacteria or their descendants, plastids at species-dependent levels. We investigated the physiological significance of the intrinsic SQDG content in the cyanobacterium *Synechococcus elongatus* PCC 7942, with the use of its mutant, in which the genes for SQDG synthesis, *sqdB* and *sqdX*, were overexpressed. The mutant showed a 1.3-fold higher content of SQDG (23.6 mol% relative to total cellular lipids, cf., 17.1 mol% in the control strain) with much less remarkable effects on the other lipid classes. Simultaneously observed were 1.6- to 1.9-fold enhanced mRNA levels for the genes responsible for the synthesis of the lipids other than SQDG, as if to compensate for the SQDG overproduction. Meanwhile, the mutant showed no injury to cell growth, however, cell length was increased (6.1 ± 2.3, cf., 3.8 ± 0.8 μm in the control strain). Accordingly with this, a wide range of genes responsible for cell division were 1.6–2.4-fold more highly expressed in the mutant. These results suggested that a regulatory mechanism for lipid homeostasis functions in the mutant, and that SQDG has to be kept from surpassing the intrinsic content in *S. elongatus* for repression of the abnormal expression of cell division-related genes and, inevitably, for normal cell division.

1. Introduction

Sulfoquinovosyl diacylglycerol (SQDG) is present in oxygenic photosynthetic organisms from cyanobacteria to their postulated descendants, plastids of plants, and contributes to construction of their membrane systems including thylakoid membranes, together with two galactolipids, monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG), and a sole phospholipid, phosphatidylglycerol (PG) [1–3]. Exceptionally, SQDG, distinct from other lipid classes, is absent in *Gloeobacter violaceus* PCC 7421, a cyanobacterium, that is regarded as the most primitive of extant cyanobacteria based on the molecular phylogenetic analysis of the 16s rRNA gene sequence, and correspondingly possesses no thylakoid membrane with a photosynthetic electron transport system functioning in cell membranes [4–6]. SQDG thus seems to have appeared later during the evolution of cyanobacteria than the other three lipids did [7].

As regards the electrochemical properties of polar head groups, SQDG is similar to PG in the possession of a negatively charged head group, but is distinct from MGDG and DGDG that contain non-charged ones [1]. The content of SQDG relative to total lipids in cyanobacterial cells or to those in plant plastids depends on the species and environmental conditions even in the same species (Fig. 1). SQDG amounts to ca. 10–20 mol% in the cells of freshwater and/or coastal cyanobacteria such as *Synechococcus elongatus* PCC 7942 and *Synechococcus* sp. PCC 7002 [7–10], and to ca. 2 to >20 mol% in plastids of red and green algae, including *C. reinhartdii* [11–14], and in those of seed plants such as *A. thaliana* [15,16]. However, ambient stress conditions such as phosphorus (P)- or sulfur (S)-depletion cause changes in the SQDG content (Fig. 1): P-depletion in *S. elongatus* PCC 7942, e.g., leads to an increase of the

Abbreviations: Chl *a*, chlorophyll *a*; DG, diacylglycerol; DGDG, digalactosyl diacylglycerol; MGDG, monogalactosyl diacylglycerol; PG, phosphatidylglycerol; PBS, phycobilisome; SQDG, sulfoquinovosyl diacylglycerol.

* Corresponding author. School of Life Sciences, Tokyo University of Pharmacy and Life Sciences, Horinouchi 1432-1, Hachioji, Tokyo 192-0392, Japan.
E-mail address: nsato@ls.toyaku.ac.jp (N. Sato).

http://dx.doi.org/10.1016/j.bbrc.2017.04.129
0006-291X/

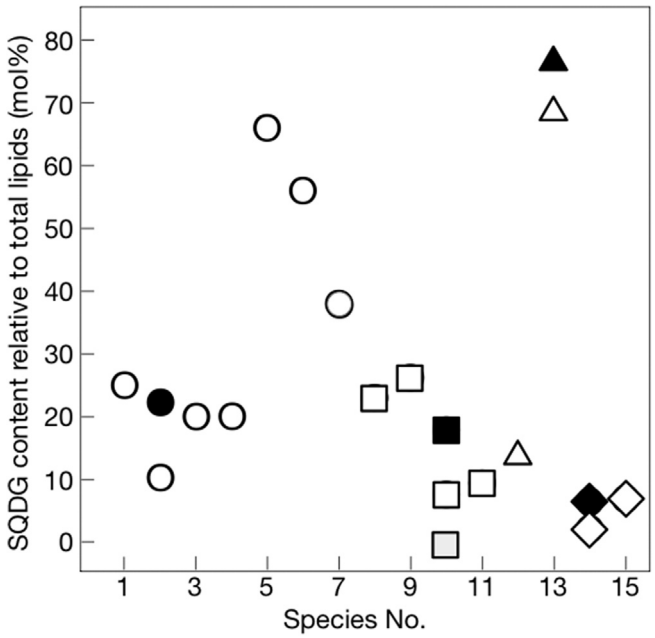

Fig. 1. SQDG contents in cellular lipids of cyanobacteria and in plant plastid lipids. Species 1–7 are cyanobacteria (circles), whereas species 8 and 9, 10 and 11, 12 and 13, and 14 and 15 belong to red algae (squares), green algae (squares), diatoms (triangles), and seed plants (diamonds), respectively, as described below. 1, *Synechocystis* sp. PCC 6803 [8]. 2, *S. elongatus* [9]. 3, *Trichodesmium erythraem* [10]. 4, *Synechococcus* sp. PCC 7002 [7]. 5, *Prochlorococcus* MED4 [10]. 6, *Synechococcus* WH8113 [10]. 7, *Synechococcus* WH8101 [10]. 8, *Polysiphonia lanosa* [11]. 9, *Cyanidioschyzon merolae* [12]. 10, *C. reinhardtii* [13,17]. 11, *Parietochloris incisa* [14]. 12, *Skeletonema costatum* [19]. 13, *Thalassiosira pseudonana* [18]. 14, *Arabidopsis thaliana* [15]. 15, *Spinacia oleracea* [16]. Shown are the SQDG contents relative to total cellular lipids in cyanobacteria and those relative to total plastid lipids, i.e., the summed contents of MGDG, DGDG, SQDG, and PG. The SQDG contents relative to total plastid lipids were estimated on the basis of data concerning cellular lipid compositions in plants including algae, and on that of data concerning thylakoid membranes in *S. oleracea*. Open symbols, non-stressed conditions. Closed symbols, phosphate-limited conditions. Grey square, sulfur-limited conditions.

SQDG content from 10 to >20 mol% with a concomitant decrease of PG, which would reflect a decrease of the P-quota due to substitution of a non-phosphorus lipid, SQDG, for PG [9]. On the contrary, sulfur-starvation induces almost complete degradation of SQDG in *C. reinhardtii* with a concomitant increase of PG [17]. Meanwhile, absolute marine cyanobacteria including *Synechococcus* and *Prochlorococcus* species, or a marine diatom, *Thalassiosira pseudonana*, which inhabit oceans with severely limited P, generally accumulate SQDG to as high as >50 mol% in total lipids of cyanobacterial cells or in those of plastid ones (Fig. 1) [10,18]. These observations imply that the extent of the contribution of SQDG synthesis to the construction of membrane systems has become diversified depending on the species, e.g., on their respective prerequisites for eco-physiological adaptation.

To answer the question of why SQDG has been conserved throughout the evolution of oxygenic photosynthetic organisms, the physiological roles of SQDG have been investigated in several species through the generation and characterization of mutants deficient in SQDG synthesis of cyanobacteria, *S. elongatus* [9], *Synechocystis* sp. PCC 6803 [8], and *Synechococcus* sp. PCC 7002 [7], a green alga, *Chlamydomonas reinhardtii* [20], and a seed plant, *Arabidopsis thaliana* [15]. Despite the evolutionary conservation of SQDG, it has been clarified that SQDG plays not common, but, if any, species-dependent roles in these organisms under normal growth conditions: as regards photosynthesis, SQDG is necessary for full functioning of the PSII complex in *Synechocystis* sp. PCC 6803 and *C. reinhardtii*, but not in *S. elongatus*, *Synechococcus* sp. PCC 7002, or *A. thaliana* [reviewed in 21]. An extreme observation was the essentiality of SQDG for cell growth in *Synechocystis* sp. PCC 6803, but not in other organisms including *S. elongatus* and *Synechococcus* sp. PCC 7002, which could be explained by the specific requirement of SQDG in *Synechocystis* among cyanobacteria for progression of the cell cycle, in particular, for DNA synthesis [8,22].

In each case above, however, the defective phenotype caused by the loss of SQDG synthesis only indicate whether or not the presence of SQDG is physiologically important, but does not lead to a comprehensive understanding of the physiological significance of the intrinsic SQDG content that depends on the species. In particular, no information has so far been obtained as to whether or not overproduction of SQDG has an impact on oxygenic photosynthetic organisms. In cyanobacteria, SQDG is synthesized through two successive reactions catalyzed by UDP-sulfoquinovose and SQDG synthases, which are coded by the *sqdB* and *sqdX* genes, respectively [1]. *S. elongatus* contains these two genes as a probable *sqdBX* operon [23]. In this study, we overexpressed *sqdBX* in *S. elongatus* for investigation of the effects of over-produced SQDG on the physiological processes in the cells.

2. Materials and methods

2.1. Cyanobacterial strains and growth conditions

The wild type (WT) cells of *S. elongatus* were cultured at 30 °C in a tube containing BG11 medium, with illumination (10 W m^{-2}) and aeration, until the OD_{730} value of the culture became ca. 1.0 for transformation [24]. Meanwhile, BXOE and EMP cells (see below) were cultured under the same conditions, but with the exception of spectinomycine addition (20 μg ml^{-1}). The OD_{730} value, and the chlorophyll *a* (Chl *a*) and phycobilisome (PBS) contents in the cultures were measured with a spectrophotometer (DU 640, Beckman) [24]. The BXOE and EMP cells that were cultured until the OD_{730} value became ca. 1.0 were collected by centrifugation at 3000×*g* for 15 min for storage at −80 °C until use for lipid or mRNA analysis.

2.2. Transformation of S. elongatus

A DNA fragment covering the coding regions of *sqdB* and *sqdX*, which are aligned in tandem in the genome of *S. elongatus*, was amplified by PCR, with KOD-Plus, primer set 1 (Table S1), and genomic DNA of *S. elongatus* as a template. The amplified DNA sequence of *sqdB-sqdX* was ligated to the *Sma*I site of the pAM1044 vector. The resultant plasmid (pBXOE) was introduced into cells of *S. elongatus* for integration of the *sqdB-sqdX* sequence into a neutral site in the genome through homologous recombination and its expression under the control of the strong constitutive promoter conII [25]. The transformant (BXOE) that gained the spectinomycine-resistance phenotype was selected. Otherwise, the empty vector was introduced into cells of *S. elongatus*, a transformant (EMP) that showed spectinomycine-resistance being generated.

2.3. Lipid analysis

Lipid analysis was performed as previously described [24]. Total lipids were extracted from cells, and thereafter separated into individual lipid classes by TLC. The spots of individual lipid classes were used for the preparation of fatty acid methyl esters for analysis by capillary GLC.

Please cite this article in press as: N. Sato, et al., Disturbance of cell-size determination by forced overproduction of sulfoquinovosyl diacylglycerol in the cyanobacterium *Synechococcus elongatus* PCC 7942, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.04.129