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Review

Type A and type B monogalactosyldiacylglycerol synthases are spatially and functionally separated in the plastids of higher plants

Koichi Kobayashi [a,c], Yuki Nakamura [b,c], Hiroyuki Ohta [d,e,*]

[a] Department of General Systems Studies, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan
[b] Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, Singapore 117604, Republic of Singapore
[c] Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259-B-65 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan
[d] Center for Biological Resources and Informatics, Tokyo Institute of Technology, 4259-B-65 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan
[e] Research Center for the Evolving Earth and Planets, Tokyo Institute of Technology, 4259-B-65 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan

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ABSTRACT

Mono- and digalactosyldiacylglycerol (MGDG and DGDG, respectively) constitute the bulk of membrane lipids in plant chloroplasts. The final step in MGDG biosynthesis occurs in the plastid envelope and is catalyzed by MGDG synthase. In *Arabidopsis*, the three MGDG synthases are classified into type A (atMGD1) and type B MGD isoforms (atMGD2 and atMGD3). atMGD1 is an inner envelope membrane-associated protein of chloroplasts and is responsible for the bulk of galactolipid biosynthesis in green tissues. MGD1 function is indispensable for thylakoid membrane biogenesis and embryogenesis. By contrast, type B atMGD2 and atMGD3 are localized in the outer envelopes and have no important role in chloroplast biogenesis or plant development under nutrient-sufficient conditions. These type B MGD genes are, however, strongly induced by phosphate (Pi) starvation and are essential for alternative galactolipid biosynthesis during Pi starvation. MGD1 gene expression is up-regulated by light and cytokinins. By contrast, Pi starvation-dependent expression of atMGD2/3 is suppressed by cytokinins but induced through auxin signaling pathways. These growth factors may control the functional sharing of the inner envelope pathway by atMGD1 and the outer envelope pathway by atMGD2/3 according to the growth environment.

1. Introduction

Life in all organisms relies on lipid bilayer cell membranes, which separate the interior of a cell from its environment [65]. In eukaryotic organisms, the compartmentalization of cells by biological membranes is an indispensable system for many cellular activities. In the membranes of animals and yeasts, glycerolipids that carry phosphate (Pi) in their head group are the most abundant polar lipid class. Likewise, these phospholipids are also the predominant components of most subcellular membranes such as the plasma membrane, peroxisomal membranes, endoplasmic reticulum, and mitochondrial membranes in higher plants [2,26,28]. In plastids, however, it is notable that the non-phosphorous glycerogalactolipids, monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), are the main constituents of the membrane lipids. MGDG accounts for approximately 50% of thylakoid membrane lipids, and together with DGDG, which constitutes up to 25 mol% of the membrane lipids, represents the bulk of the photosynthetic membranes in plant chloroplasts [8]. The anionic glycerolipids, sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG), are also typical components of thylakoid lipids, which together account for approximately 15 mol% of the membrane lipids in plant chloroplasts [8]. Cyanobacteria, which possess thylakoid membranes and perform oxygenic photosynthesis in a manner similar to the chloroplasts of seed plants, have a membrane lipid composition similar to the thylakoids of plant chloroplasts. For example, *Synechocystis* sp. PCC 6803 contains 59% MGDG, 17% DGDG, 16% SQDG, and 8% PG in its membranes [72]. The similarity in glycerolipid composition between cyanobacteria and chloroplasts can be explained according to the endosymbiotic hypothesis [48].

Abbreviations: DAG, *sn*-1:2-diacylglycerol; DGD, digalactosyldiacylglycerol synthase; DGDG, digalactosyldiacylglycerol; LHC, light harvesting chlorophyll–protein complex; MGD, monogalactosyldiacylglycerol synthase; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PG, phosphatidylglycerol; Pi, phosphate; PS, photosystem; SQDG, sulfoquinovosyldiacylglycerol.

* Corresponding author. Center for Biological Resources and Informatics, Tokyo Institute of Technology, 4259-B-65 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan. Tel.: +81 45 924 5736; fax: +81 45 924 5823.
E-mail address: ohta.h.ab@m.titech.ac.jp (H. Ohta).

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The high abundance of galactolipids in chloroplasts and cyanobacteria, and their absence in most non-photosynthetic organisms, suggests the special importance of galactolipids for oxygenic photosynthesis. On the other hand, MGDG-accumulating *Escherichia coli* display a rough and scratched cell membrane surface and have defects in cell division [15], suggesting that MGDG is toxic to *E. coli*. MGDG has a small galactose head group and broadened polyunsaturated fatty acid tails, which together give this molecule a cone-like shape and the ability to induce curvature in lamellar membranes [49]. In vitro assembly analysis revealed that MGDG is responsible for lamellar organization of the thylakoid membranes through its association with light harvesting chlorophyll–protein complex (LHC) II [64]. In contrast to MGDG, which forms non-bilayer hexagonal phases, DGDG has a more cylindrical shape and forms bilayer lamellar phases in mixtures with water. These unique galactolipid characteristics may be important for the organization of highly stacked thylakoid membranes [39]. Indeed, the ratio of bilayer to non-bilayer-forming lipids is crucial for maintaining membrane stability and the functional activity of membrane proteins [73]. A number of studies have shown that galactolipids are required not only as bulk constituents of photosynthetic membranes, but also for the photosynthetic reactions. X-ray crystallographic analyses of photosynthetic proteins in cyanobacteria demonstrated that MGDG is associated with the core of the reaction center of both photosystem (PS) I and PS II and may be important for electron transfer in PS complexes [25,42,43]. Moreover, MGDG has a crucial role in the efficiency of photosynthetic reactions under high light conditions by maintaining the appropriate proton motive force in thylakoids [3]. DGDG has also been found in the crystallized PS II complex and in LHC II [41,42,66]. Analyses of a DGDG-deficient *Arabidopsis* mutant (*dgd1*) indicated that DGDG plays a crucial role in PS II activity and stabilization of trimeric LHC II [11,22,67]. The *dgd1* mutant also demonstrated the importance of DGDG for the stability and activity of the PS I complex [19,23]. A *Synechocystis dgdA* mutant that completely lacks DGDG revealed that DGDG is required for stabilization of the oxygen-evolving complex in PS II [60]. Taken together, these data indicate the direct involvement of MGDG and DGDG in the photosynthetic reactions of oxygenic photosynthetic organisms.

Besides the functions of glycolipids in photosynthesis, these nonphosphorous lipids are involved in membrane maintenance during Pi deficiency. Under Pi-starved conditions, phospholipid degradation is activated to sustain other cellular processes such as nucleic acid biosynthesis, presumably by economizing Pi in the membranes [1,16,51]. Compensation for the reduced PG with SQDG during Pi starvation has been reported in *Rhodobacter sphaeroides* [7], *Synechococcus* sp. PCC7942 [18], *Chlamydomonas reinhardtii* [58], and *Arabidopsis thaliana* [14,80]. In higher plants, accumulation of high levels of DGDG in response to Pi deficiency occurs in plastidic membranes or extraplastidic membranes [21] such as the plasma [2], tonoplast [1], and mitochondrial membranes [26], although DGDG is localized exclusively in plastids under nutrient-sufficient conditions. These observations suggest that DGDG replaces phospholipids as the major membrane component under Pi-limited growth conditions. In contrast, no MGDG increase is observed in extraplastidic membranes during Pi starvation, suggesting that bilayer-forming DGDG rather than non-bilayer-forming MGDG is important for the complementation of phospholipids in the extraplastidic membranes of plant cells [21]. The importance of DGDG during Pi starvation was also reported in *Synechocystis* sp. PCC 6803 [5]. These results suggest that the utilization of galactolipids in the thylakoid membrane is an adaptive mechanism for phosphate-limited conditions [10].

In higher plants, galactolipid biosynthesis occurs in the plastid envelope [12]. MGDG biosynthesis is catalyzed by MGDG synthase (UDP-galactose: 1,2-diacylglycerol 3-[α]-D-galactosyltransferase, EC 2.4.1.46), which transfers a galactosyl residue from UDP-galactose to the *sn*-3-position of *sn*-1,2-diacylglycerol (DAG) [61]. DGDG is synthesized by galactosylation of MGDG [29,30]. Thus, MGDG synthase is the key enzyme for the biosynthesis of both of these galactolipids, and hence, the biogenesis of chloroplast membranes. Despite the importance of MGDG synthase for plants, purification of the enzyme is extremely difficult due to its very low abundance even in photosynthetic tissues [45]. However, since an MGDG synthase protein and its corresponding gene were identified in cucumber cotyledons by Shimojima et al. [61], significant progress has been made in this field at the molecular level. Here, we review recent findings concerning the physiological roles and regulation of plant MGDG synthases in response to various growth conditions and developmental stages.

2. Identification of MGDG synthase in seed plants

2.1. Purification of the enzyme that catalyzes the final step of MGDG synthesis in seed plants

Initial efforts to isolate the enzyme that catalyzes the final step of MGDG synthesis in plants were made by two groups using the chloroplast envelope fraction. Teucher and Heinz proposed that a 22-kDa polypeptide was associated with the activity [69]. In contrast, Maréchal et al. identified a 19-kDa polypeptide as the enzyme [44]. In each case, the amount of purified protein was too low to determine the amino acid sequences because there was a difficulty to prepare a large amount of chloroplast envelope membranes containing the MGDG synthase from leaf samples. Shimojima et al. started the purification of MGDG synthase using cucumber cotyledons [61], which showed the highest MGDG synthase activity among all plant samples investigated after light irradiation [55]. The enzyme was purified from a chloroplast envelope-enriched microsome fraction as a 47 kDa protein [54,61]. The corresponding gene (*csMGD1*) was cloned from a cDNA library of cucumber cotyledons according to the partial amino acid sequence of the purified enzyme. Transgenic *E. coli* expressing the cloned MGD gene showed MGDG synthase activity and a substantial accumulation of MGDG in its membranes, confirming that the cloned gene codes for a functional MGDG synthase [61].

Identification of the cucumber MGD gene enabled us to expand the molecular analysis of MGD genes to other plants. Miège et al. identified a spinach MGDG synthase (soMGD1) that is homologous to csMGD1 and has 73.4% sequence similarity [46]. soMGD1, which possesses a putative transit peptide to chloroplasts, was localized within a leaflet of the inner envelope membrane in spinach chloroplasts and presumably forms homodimers. In *Arabidopsis*, in which genomic sequencing has been fully completed, three functional MGDG synthases have been identified and designated atMGD1, atMGD2, and atMGD3 [4,46]. Based on their amino acid identities, these three isoforms can be phylogenetically classified into two groups, type A (atMGD1) and type B (atMGD2 and atMGD3). Type A and type B have greatly different enzymatic properties and physiological roles in membrane homeostasis and plant development as described below.

2.2. The characteristics of the two types of MGDG synthases in Arabidopsis

atMGD1 shares more than 80% amino acid homology with csMGD1 and soMGD1 and possesses a transit peptide in its N-terminus as other type A MGD do. By contrast, atMGD2 and atMGD3 show relatively lower amino acid identities to atMGD1 (about 60%) and have no putative transit peptides. Immunochemical studies as