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Biosynthesis of unnatural glycolipids possessing diyne moiety in the acyl chain in the green sulfur photosynthetic bacterium *Chlorobaculum tepidum* grown by supplementation of 10,12-heptadecadiynic acid

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ABSTRACT

Unnatural glycolipids possessing the diyne moiety in their acyl groups were successfully biosynthesized in the green sulfur photosynthetic bacterium *Chlorobaculum* (*Cba.*) *tepidum* by cultivation with supplementation of 10,12-heptadecadiynic acid. Monogalactosyldiacylglycerol (MGDG) and rhamnosylgalactosyldiacylglycerol (RGDG) esterified with one 10,12-heptadecadiynic acid were primarily formed in the cells, and small amounts of glycolipids esterified with the two unnatural fatty acids can also be detected. The relative ratio of these unnatural glycolipids occupied in the total glycolipids was estimated to be 49% based on HPLC analysis using a evaporative light scattering detector. These results indicate that the acyl groups in glycolipids, which play important roles in the formation of extramembranous antenna complexes called chlorosomes, can be modified *in vivo* by cultivation of green sulfur photosynthetic bacteria with exogenous synthetic fatty acids. Visible absorption and circular dichroism spectra of *Cba. tepidum* containing the unnatural glycolipids demonstrated the formation of chlorosomes, indicating that the unnatural glycolipids in this study did not interfere with the biogenesis of chlorosomes.

1. Introduction

Photosynthetic light-harvesting complexes capture the sunlight energy and transfer it to the reaction center complexes in the early stage of photosynthetic events. In most light-harvesting complexes, photosynthetic pigments such as chlorophylls (Chls), bacteriochlorophylls (BChls), and carotenoids are embedded in the protein matrix [1]. The only exception is extramembranous antenna complexes of green photosynthetic bacteria called chlorosomes [2–5]. Chlorosomes are ellipsoidal particles with the dimensions of approximately 100–150 nm length, 30–50 nm width, and 10–25 nm height. The huge number of BChls *c*, *d*, *e*, and *f* are densely packed and assembled with no help of proteins in the interior of chlorosomes [6,7]. The BChl self-aggregates are surrounded by a lipid layer on chlorosomes. The specific interactions among chlorosomal BChl pigments, namely the coordination bond of the 3^1-hydroxy group of one BChl with the central magnesium in another BChl and the hydrogen bond of the coordinated 3^1-hydroxy group with the 13-keto group in a third BChl, play crucial roles in the pigment self-assemblies in chlorosomes [8,9]. Such unique architecture has attracted considerable attentions in photobiology and photobiophysics.

The major components of the envelop of chlorosomes are glycolipids and membranous proteins called Csm proteins [3,5,10–13]. The green sulfur photosynthetic bacterium *Chlorobaculum* (*Cba.*) *tepidum* has two types of glycolipids, namely monogalactosyldiacylglycerol (MGDG) and disaccharide-type rhamnosylgalactosyldiacylglycerol (RGDG), both of which are attached with various acyl groups (Fig. 1). In addition, *Cba. tepidum* possesses ten kinds of Csm proteins, whose roles in chlorosomes have been studied by means of biochemical and molecular genetic techniques [14–17]. In contrast to the extensive researches on the Csm proteins, detailed contributions of glycolipids for chlorosomes have not been unraveled yet. One possible reason for little information of glycolipids in chlorosomes comes from difficulties in separation and characterization of glycolipids from green photosynthetic bacteria. Recently, Tamiaki and coworkers have performed precise analysis of glycolipids in green photosynthetic bacteria to

Abbreviations: BChl, bacteriochlorophyll; BChl c_F, bacteriochlorophyll *c* esterified with farnesol; *Cba.*, *Chlorobaculum*; ELSD, evaporative light scattering detector; ESI, electrospray ionization; MGDG, monogalactosyldiacylglycerol; RGDG, rhamnosylgalactosyldiacylglycerol

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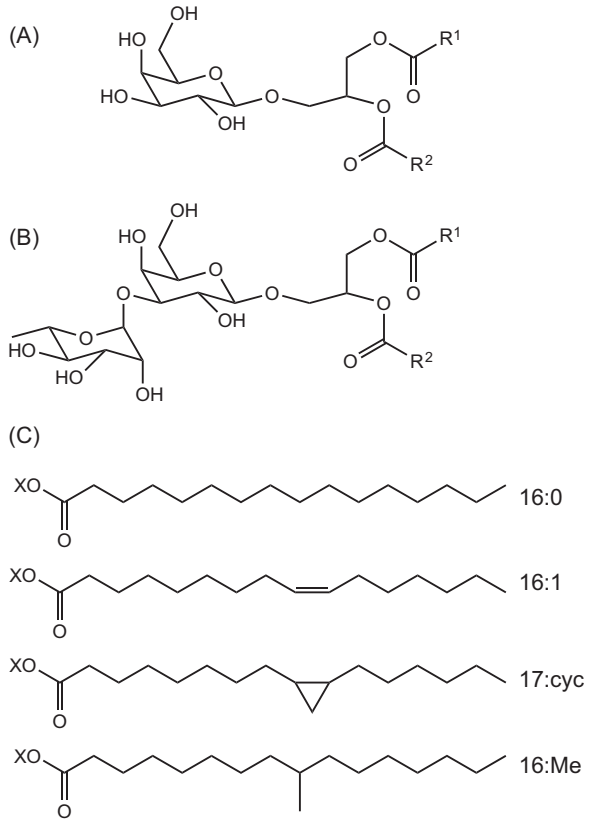

Fig. 1. The molecular structures of MGDG (A) and RGDG (B) found in chlorosomes of *Cba. tepidum*. Hydrocarbons in the acyl chains are indicated by R^1 and R^2 in A and B. The structures of major acyl chains (R^1 and R^2) in glycolipids are shown in C, where the esterifying alcoholic parts are indicated by X.

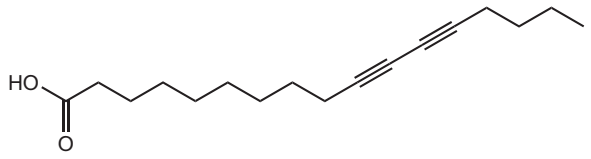

Fig. 2. Molecular structure of 10,12-heptadecadiynic acid supplemented in the liquid cultures of *Cba. tepidum* in this study.

overcome these problems [18–22]. Another problem would originate from difficulties in alteration of glycolipids in green photosynthetic bacteria. There is little information, to our best knowledge, on the genetic approach to glycolipids in green photosynthetic bacteria [23]. The methodology for *in vivo* modification of glycolipids in green photosynthetic bacteria will be a clue to investigation of the supramolecular structure and biogenesis of chlorosomes. In this study, we first report modification of the acyl moieties in glycolipids of *Cba. tepidum* using their biosynthetic reactions by supplementation of a commercially available fatty acid 10,12-heptadecadiynic acid, which has a diyne moiety in the middle of the hydrocarbon chain (the molecular structure is shown in Fig. 2).

2. Experimental

2.1. Apparatus

Analysis of glycolipids by a HPLC system equipped with an evaporative light scattering detector (ELSD) was performed with a Shimadzu LC-20AT pump and an ELSD-LT II detector by the control of column temperature with a Shimadzu CTO-20AC column oven. HPLC analysis of BChl *c* was carried out with a Shimadzu LC-20AT pump and an SPD-M20A detector. Liquid chromatography-mass spectrometry (LC-MS) was done with a Shimadzu LCMS-2020 system equipped with an electrospray ionization probe (ESI). Visible absorption and circular dichroism (CD) spectra were measured with a Shimadzu UV-2450 spectrophotometer and a JASCO J-820 spectropolarimeter, respectively.

2.2. Cultivation

Pre-cultured cells (1 mL) of the green sulfur photosynthetic bacterium *Cba. tepidum* ATCC 49652 were inoculated into a freshly prepared liquid medium (*ca.* 650 mL), in which 10 mg of 10,12-heptadecadiynic acid (Tokyo Chemical Industry, Co., Ltd.) was supplemented. Then, *Cba. tepidum* was grown in the liquid medium by continuous irradiation with fluorescence lamps (13 μmol s^{-1} m^{-2}) at 42 °C for 3 days [24–26].

2.3. Extraction and analysis of glycolipids

Glycolipids were extracted from the harvested cells with a mixture of 0.1 M acetic acid, methanol, and chloroform, and purified from the crude extracts by silica-gel chromatography according to the previous reports [18–22]. Glycolipids obtained were analyzed by ELSD-HPLC using a reverse-phase column Cosmosil $5C_{18}$-AR-II (4.6 mm i.d.×250 mm) with acetone/25 mM ammonium acetate (pH 6.7) (85/15, vol/vol) at the flow rate of 0.5 mL min^{-1}. Glycolipids were assigned by LC-MS as well as the elution patterns reported previously [18–22].

2.4. Extraction and analysis of BChl c

BChl *c* was extracted from the harvested cells with methanol/acetone (1/1, vol/vol), followed by filtration. The organic solutions containing the extracted pigments were diluted with diethyl ether, washed with NaCl-saturated water, and dried over anhydrous Na_2SO_4, followed by evaporation. BChl *c* obtained was analyzed by HPLC using a reverse-phase column Cosmosil $5C_{18}$-AR-II (6 mm i.d.×250 mm) with methanol/water (95/5, vol/vol) at the flow rate of 1.0 mL min^{-1} [24–27].

3. Results and discussion

3.1. Compositions of glycolipids

Glycolipids were extracted from *Cba. tepidum* cells, which were grown under the normal conditions and by supplementation of 10,12-heptadecadiynic acid, and analyzed by ELSD-HPLC. Hereafter the following abbreviations are used for naturally occurring fatty acids at the main chain of glycolipids: palmitic (16:0), palmitoleic (16:1), methylated palmitic (16:Me), and methylene-bridged palmitoleic (17:cyc) acids. Fig. 3 shows typical elution patterns of glycolipids extracted from *Cba. tepidum* grown under the normal conditions and by supplementation of 10,12-heptadecadiynic acid. *Cba. tepidum* grown under the normal conditions possessed six major glycolipids, which eluted from 18 to 30 min under the present HPLC conditions (fractions 1–6 in Fig. 3A). These natural glycolipids were analyzed by LC-MS: the results of their online ESI-MS spectrometry are summarized in Table 1. The present LC-MS analysis and the elution order in the previous reports [18–22] allowed us to assign the naturally occurring glycolipids (fractions 1–6) to be RGDG (16:1,16:0), MGDG (16:1,16:0), RGDG (17:cyc,16:0), MGDG (17:cyc,16:0), MGDG (16:0,16:0), and MGDG (16:Me,16:0), respectively.

Supplementation of 10,12-heptadecadiynic acid in the liquid medium of *Cba. tepidum* produced novel glycolipids, which were observed from 7 to 13 min in the ELSD-HPLC chromatogram (fractions 1′–6′ in Fig. 3B). The molecular ion peaks of these novel glycolipids were observed at *m/z* 906.6, 760.5, 912.6, 766.6, 900.7, and 754.6,