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Cyclopropane-ring formation in the acyl groups of chlorosome glycolipids is crucial for acid resistance of green bacterial antenna systems

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ABSTRACT

Green photosynthetic bacteria have unique light-harvesting antenna systems called chlorosomes. *Chlorobaculum tepidum*, a model organism of the bacteria, biosynthesized monogalactosyl- and rhamnosylgalactosyldiacylglycerides possessing a methylene-bridged palmitoleyl group characterized by a *cis*-substituted cyclopropane ring as the dominant glycolipids of its chlorosome surface. The formation of the cyclopropane ring was chemically inhibited by supplementation of sinefungin, an analog of *S*-adenosyl-L-methionine, into the bacterial cultivation. The presence of the cyclopropane ring reinforced acid resistance of the light-harvesting chlorosomes and suppressed acidic demetalation (pheophytinization) of bacteriochlorophyll-*c* pigments constructing the core part of chlorosomes. The ring-formation would represent direct and post-synthetic modifications of chlorosome membrane properties and was tolerant of acidic environments.

1. Introduction

'Chlorosomes' are submicrometer-sized, extra-membraneous light-harvesting antenna systems that characteristically contain huge quantities of bacteriochlorophyll(BChl)-*c*/*d*/*e*/*f* molecules as self-aggregated forms (>200,000 molecules per single chlorosome).[1–3] They are surrounded by a protein containing lipid monolayer membrane, that is, micelle-like architectures.[4,5] Chlorosomes have been found in the following members of green photosynthetic bacteria grown optimally in a wide range of temperatures (30–55 °C): green sulfur bacteria, green filamentous anoxygenic phototrophs (previously referred to as green non-sulfur bacteria) and one species from the phylum *Acidobacteria*, '*Candidatus* Chloracidobacterium thermophilum'.[6,7]

Recently, we reported the structures and compositions of glycolipids in chlorosome membranes of various green photosynthetic bacteria at the molecular level, and proposed a temperature-tolerant strategy acquired by the organisms which grew over the aforementioned broad range of temperatures from the viewpoint of the chain-length of acyl groups in glycolipids.[8] As a result, dependence of the chain-length of their acyl groups upon bacterial cultivation temperatures was clearly observed. The organisms with their optimal temperatures of 30, 45 and 55 °C dominantly accumulated glycolipids possessing the acyl chains in the range of C_{15}–C_{16}, C_{16}–C_{17} and C_{18}–C_{20}, respectively. Interestingly, the green sulfur bacterium *Chlorobaculum* (*Cba.*) *tepidum* with an optimal temperature of 45 °C preferentially biosynthesized unique glycolipids with a methylene-bridged palmitoleyl group (C_{17}) containing a *cis*-substituted cyclopropane ring in their sn-1 acyl group (see Graphical abstract).[8,9] Such glycolipids played a heat-protective function for the light-harvesting chlorosomes of *Cba. tepidum*, based on temperature-dependent changes in electronic absorption spectra of the chlorosomes whose glycolipids were modified by changing cultivation conditions (with or without cyclopropane-ring containing glycolipids).[10]

The presence of cyclopropane ring-containing lipids, especially in phospholipids, was reported for many bacteria,[11] and a strong correlation between the acid survival of *Escherichia coli* to a rapid and drastic decrease in pH and the content of such lipids present in the cell membranes was demonstrated.[12] It was also proposed that the cyclopropane ring was created by stereospecific insertion

Abbreviations: BChl, bacteriochlorophyll; BPhe, bacteriopheophytin; *Cba.*, *Chlorobaculum*; DLS, dynamic light scattering; ELSD, evaporative light scattering detector; ESI, electrospray ionization; MGDG, monogalactosyldiacylglyceride; PAGE, polyacrylamide gel electrophoresis; RGDG, rhamnosylgalactosyldiacylglyceride; SAM, *S*-adenosyl-L-methionine; SDS, sodium dodecylsulfate.

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of a methylene group, derived from the reactive methyl group of *S*-adenosyl-L-methionine (SAM), to the C=C double bond of unsaturated fatty acids (usually palmitoleyl or oleyl).[11] Notably, the methylene group was transferred to mature lipid molecules already present in membranes and did not involve free fatty acids or intermediates in the lipid biosynthesis. Its production, moreover, typically occurred at the onset of the stationary phase of bacterial cultivation. Thus, the formation of a cyclopropane ring in lipid molecules is considered to be a direct and post-synthetic modification of bacterial membrane properties.

Here, we performed chemical inhibition of the cyclopropane-ring formation in the acyl groups of chlorosome glycolipids by supplementation of sinefungin (adenosyl ornithine), an analog of SAM whose reactive CH_3 was replaced with NH_2, to the culture of *Cba. tepidum*. In order to investigate the role of the cyclopropane-ring in chlorosome glycolipid molecules in addition to their previously demonstrated heat-protective function,[10] acid resistance of the light-harvesting chlorosomes was demonstrated in terms of demetalation of BChl-*c* pigments to produce bacteriopheophytin(BPhe)-*c* inside chlorosomes.

2. Results and discussion

2.1. Inhibition of the cyclopropane-ring formation by chemical supplementation

Figure 1 shows the results of inhibition of the cyclopropane-ring formation in chlorosome glycolipids for the green sulfur bacterium *Cba. tepidum* by supplementation of a chemical inhibitor, sinefungin, at the concentration of 0–20 μM (see also Graphical abstract). At less than the concentration of 20 μM, no inhibition of the bacterial growth was confirmed as seen in Figure 1a. As the formation occurred at the onset of the stationary phase of bacterial cultivation confirmed in the previous study,[10] *Cba. tepidum* cultured with or without sinefungin were harvested at the stationary phase (96 h) and their light-harvesting chlorosomes were isolated from the cultured cells.

Figure 1b shows the HPLC profiles obtained by an evaporative light scattering detector (ELSD) (hereafter simply referred to as ELSD–HPLC) of glycolipids extracted from the chlorosomes of the cells cultured with supplementation of sinefungin at 0, 10 and 20 μM (from top to bottom). Several glycolipids possessing a monosaccharide (monogalactosyldiacylglyceride, MGDG) or disaccharide (rhamnosylgalactosyldiacylglyceride, RGDG) moiety as well as different acyl groups were identified. Assignments of each glycolipid are labeled in the profiles (MGDGs and RGDGs are represented by 'M' and 'R', respectively). The structures of MGDG/RGDG and their acyl groups are shown in Figure 2, together with the abbreviations of the acyl groups used in this study. Data of on-line electrospray ionization (ESI)-mass spectrometry are shown in Tables S1–S3. Characterization of glycolipids found in the profiles by $^{1}H/^{13}C$ NMR, GCMS coupled with derivation of their two acyl groups as well as site-specific hydrolysis of the sn-1 acyl chain was reported in our previous studies.[8,9]

The control chlorosomes (at 0 μM sinefungin) were dominantly composed of glycolipids with a methylene-bridged palmitoleyl group containing a characteristic cyclopropane ring, MGDG/RGDG (17:Cyc,16:0) (83.2% of the total glycolipids), in addition to glycolipids with a branched methyl group in the acyl chain, MGDG/RGDG (16:Me, 16:0). With an increase in the concentration of sinefungin, the content of the cyclopropane-ring containing glycolipids drastically decreased, and concomitantly that of the glycolipids bearing a general 9-(*Z*)-palmitoleyl group, MGDG/RGDG (16:1, 16:0), increased. Finally, at the concentration of 20 μM, the formation of the cyclopropane-ring largely inhibited [see Fig. 1b bottom].

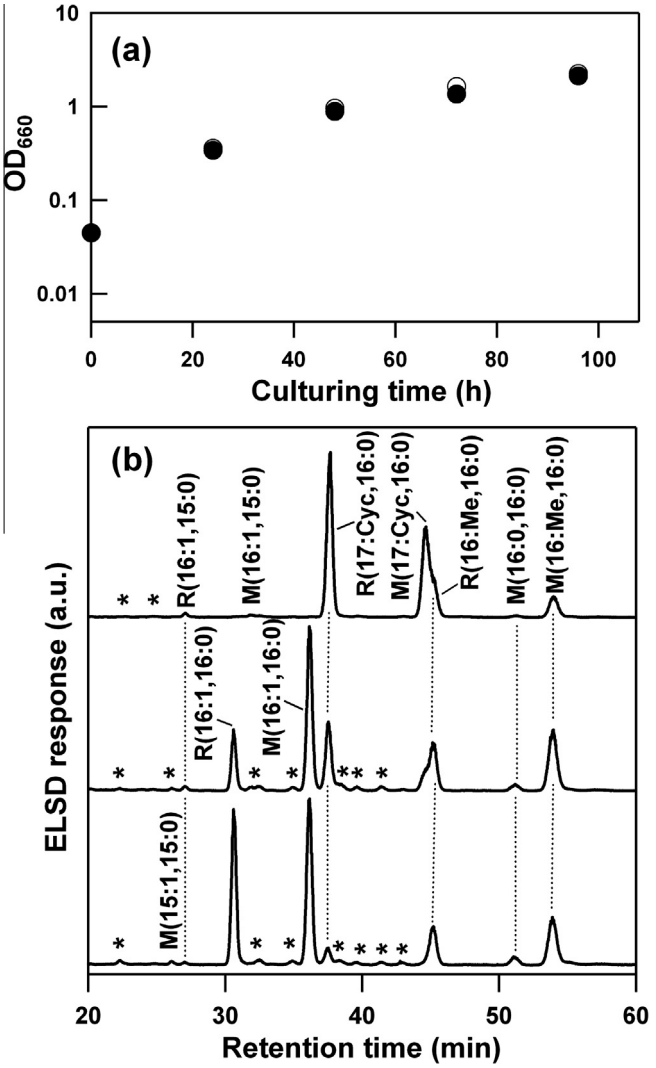

Figure 1. Inhibition of cyclopropane-ring formation in glycolipids by supplementation of sinefungin. (a) Growth profiles of *Cba. tepidum* with (closed circles, 20 μM) and without sinefungin (open circles) at its optimal 45 °C, and (b) ELSD-HPLC profiles of glycolipids extracted from the chlorosomes of *Cba. tepidum* supplemented at 0, 10 and 20 μM sinefungin (from top to bottom). Unidentified components are indicated by asterisks. MGDGs and RGDGs are represented by 'M' and 'R', respectively, and abbreviations of fatty acids are seen in Figure 2.

2.2. Acid resistance of light-harvesting chlorosomes

Three types of chlorosomes composed of modified glycolipid compositions were isolated from *Cba. tepidum* as follows: 'control chlorosomes' were isolated from the cells grown under usual cultivation conditions,[13] 'cyclopropane-ring depleted chlorosomes' from the cells cultured with supplementation of 20 μM sinefungin as mentioned above, and 'low-temperature adapted chlorosomes' from the cells cultured at a decreased temperature of 25 °C.[10] Here, we used low-temperature adapted chlorosomes as the model for most of the other green sulfur bacteria grown at their optimal temperature of 30 °C, since the composition of low-temperature adapted chlorosomes was almost identical to those of the other green sulfur bacteria.[8] The glycolipid compositions of such chlorosomes determined by peak areas of ELSD–HPLC are summarized in Table 1: control, cyclopropane-ring depleted and low-temperature adapted chlorosomes contained as their main glycolipids MGDG/RGDG (17:Cyc,16:0), MGDG/RGDG (16:1, 16:0)/(16:Me, 16:0), and MGDG (16:1, 16:0)/(16:1, 16:1), respectively. The ratios of