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Phospholipid distributions in purple phototrophic bacteria and LH1-RC core complexes

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ABSTRACT

In contrast to plants, algae and cyanobacteria that contain glycolipids as the major lipid components in their photosynthetic membranes, phospholipids are the dominant lipids in the membranes of anoxygenic purple phototrophic bacteria. Although the phospholipid compositions in whole cells or membranes are known for a limited number of the purple bacteria, little is known about the phospholipids associated with individual photosynthetic complexes. In this study, we investigated the phospholipid distributions in both membranes and the light-harvesting 1-reaction center (LH1-RC) complexes purified from several purple sulfur and nonsulfur bacteria. ^{31}P NMR was used for determining the phospholipid compositions and inductively coupled plasma atomic emission spectroscopy was used for measuring the total phosphorous contents. Combining these two techniques, we could determine the numbers of specific phospholipids in the purified LH1-RC complexes. A total of approximate 20–30 phospholipids per LH1-RC were detected as the tightly bound lipids in all species. The results revealed that while cardiolipin (CL) exists as a minor component in the membranes, it became the most abundant phospholipid in the purified core complexes and the sum of CL and phosphatidylglycerol accounted for more than two thirds of the total phospholipids for most species. Preferential association of these anionic phospholipids with the LH1-RC is discussed in the context of the recent high-resolution structure of this complex from *Thermochromatium* (*Tch.*) *tepidum*. The detergent lauryldimethylamine *N*-oxide was demonstrated to selectively remove phosphatidylethanolamine from the membrane of *Tch. tepidum*.

1. Introduction

Phospholipids and glycolipids make up the matrix of bilayer biological membranes and both of them are polar lipids. While the glycolipids are the most abundant lipid components in the photosynthetic membranes of plants, algae and cyanobacteria, phospholipids are the major lipids in the membranes of the evolutionarily more ancient anoxygenic phototrophic bacteria under standard culture conditions. In purple phototrophic bacteria, typical phospholipids include phosphatidylglycerol (PG), phosphatidylethanolamine (PE), cardiolipin (CL) and phosphatidylcholine (PC) [1,2]. Their compositions are known to be highly variable among different species and strongly dependent on environmental factors such as growth conditions and nutrient supply. PG is the only phospholipid found in the membranes of virtually all phototrophic organisms from bacteria to higher plants. PE and CL are present in most purple bacteria, although the latter was reported to be absent in some of the purple nonsulfur *Rhodobacter* (*Rba.*) species [3]. With few exceptions, PC is absent in species of the purple sulfur bacteria of the family *Chromatiaceae* but are present in all species of *Ectothiorhodospira* and most purple nonsulfur bacteria [4,5].

Despite a long history of research on the membrane lipids in purple phototrophic bacteria, most studies have been devoted to rather qualitative analyses of the lipid compositions of whole cells or membranes, the influence of the culturing conditions on the compositions, and the

Abbreviations: Alc., *Allochromatium*; *Blc.*, *Blastochloris*; CL, cardiolipin; DDM, *n*-dodecyl-β-D-maltopyranoside; GGDG, glucosylgalactosyldiacylglycerol; ICP-AES, inductively coupled plasma atomic emission spectroscopy; LDAO, lauryldimethylamine *N*-oxide; LH1, core light-harvesting complex; LH2, peripheral light-harvesting complex; OG, *n*-octyl-β-D-glucopyranoside; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; *Pha.*, *Phaeospirillum*; *Rba.*, *Rhodobacter*; RC, reaction center; *Rbl.*, *Rhodoblastus*; *Rsp.*, *Rhodospirillum*; SMA, styrene-maleic acid; *Tch.*, *Thermochromatium*

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mechanisms of the lipid biosynthesis and their regulation [1,6]. Alterations in phospholipid composition induced by oxygen and additives in the medium were extensively investigated [7,8]. However, few studies have dealt with the phospholipid composition of purified photosynthetic complexes, although it may differ significantly from that in the cell membranes. Such knowledge is essential for our understanding of the roles of specific phospholipids in controlling protein functions.

Investigations of the lipid function and interaction with photosynthetic complexes were triggered by the discovery of a tightly bound CL molecule in the crystallographic structure of the reaction center (RC) complex from *Rba. sphaeroides* [9]. The head group of the anionic CL interacts non-covalently with a number of basic amino acid residues in the M-subunit near the cytoplasmic surface of the membrane, whereas the acyl chains interact with hydrophobic regions of the protein in the membrane interior. As extensively reviewed by Jones [10], the CL molecule may have a general function in stabilization of the RC structure by strengthening the protein scaffold and filling the intra-protein cavities; this has been proposed because the CL binding site is conserved across nearly 50 species of purple bacteria. Biochemical studies suggested possible roles of the lipids(CL)–protein interactions in modulation of electron transport through quinone molecules and the presence of more than one lipid binding site in the *Rba. sphaeroides* RC [11–13]. In addition to the CL, one PC near the Q_B site and one glucosylgalactosyldiacylglycerol (GGDG) molecule were later resolved in a crystal structure of the *Rba. sphaeroides* RC [14]. A summary of the lipids reported in bacterial photosynthetic membrane complexes is given in Supporting information Table S1.

No phospholipids have been reported in the crystal structures of the RC from the bacteriochlorophyll *b*-containing purple nonsulfur bacterium *Blastochloris* (*Blc.*) *viridis*, although a diacylglycerol covalently bound to the N-terminal end of C-subunit and a monoolein in the Q_B binding site were identified [15–17]. Another crystal structure of the RC complex from the thermophilic purple sulfur bacterium *Thermochromatium* (*Tch.*) *tepidum* revealed a PE molecule bound to a site close to that of the GGDG but distinct from that of the CL in the *Rba. sphaeroides* RC [18]. In the *Tch. tepidum* RC, the position corresponding to the CL in the *Rba. sphaeroides* RC was occupied by a molecule of the detergent *n*-octyl-β-D-glucopyranoside (OG). For the peripheral light-harvesting complex (LH2) of the purple nonsulfur bacteria *Rhodoblastus* (*Rbl.*) *acidophilus* (formerly *Rhodopseudomonas acidophila*) and *Phaeospirillum* (*Pha.*) *molischianum*, no lipid molecules were resolved in the X-ray crystal structure, although some extra electron densities that could not be modeled by protein and pigment may arise from lipids or detergents as a result of exchange during the purification process [19–21]. In fact, detergent molecules were confirmed on both outer and inner surfaces of the *Rbl. acidophilus* LH2 by neutron diffraction experiments [22]. In contrast, biochemical analysis demonstrated that the purified LH2 complex from cells grown under anoxic high-light conditions contained approximately equal quantities of PE and PC as major phospholipids with CL as a minor component and no PG, although the latter was present in the cell membrane [23]. This provides an excellent example of how the phospholipid composition associated with individual complexes can differ significantly from that of cell membranes. A number of PE molecules were also identified in the crystal structure of the cytochrome bc_1 complex from *Rba. sphaeroides* [24]. Beside the detergents used for solubilization of membrane proteins, styrene-maleic acid (SMA) copolymers have been used as new tools for extracting photosynthetic membrane complexes (Table S1), which could probe the local lipid environment of a specific complex [25–27].

Recently, a high-resolution crystallographic structure of the core light-harvesting (LH1)-RC complex from *Tch. tepidum* has been determined [28], and this has provided an ideal opportunity for analyzing–both qualitatively and quantitatively–the lipid composition of this core complex. In order to assist modeling of the phospholipid molecules in the structure, it was essential to know the numbers of each phospholipid in the purified LH1-RC complex. In this study, we have measured the phospholipid compositions in both membranes and purified LH1-RC complexes from several species of purple sulfur and nonsulfur bacteria and quantified the phospholipid molecules in the purified core complexes. The phospholipid distributions in membranes and purified LH1-RC complexes were compared to show that the minor component CL in the membranes is preferentially associated with the LH1-RC complexes as the major phospholipid in both purple sulfur and nonsulfur bacteria alike. Confinement of the CL to LH1-RC has been suggested to stabilize the charge separation state in the *Rba. sphaeroides* RC [29]. Part of the result of present work was used as a reference in the assignment of the phospholipid molecules in the high-resolution crystal structure of *Tch. tepidum* LH1-RC complex.

2. Materials and methods

2.1. Growth conditions

The purple sulfur bacteria *Tch. tepidum* strain MC and *Allochromatium* (*Alc.*) *vinosum* strain D, and three purple nonsulfur bacteria *Rhodospirillum* (*Rsp.*) *rubrum* (NBRC 3986), *Rba. sphaeroides* (NBRC 12203^T) and *Blc. viridis* (DSM 133^T) were used in this study. Unless otherwise stated, all bacteria were grown anaerobically under continuously incandescent illumination by 60 W tungsten bulbs at a light intensity of 2000 lx (~40 μmol m^{-2} s^{-1}) in complete media for 7 days. The *Tch. tepidum* cells were grown at 48–49 °C and the cells of other bacteria were grown at 23–25 °C. *Tch. tepidum* cells were also grown in a growth chamber illuminated by LED lamps (peaks at 450 nm and 645 nm) at a light intensity of 30 μmol m^{-2} s^{-1} at 49 °C for 7 days [28]. The samples obtained under this illuminating condition are designated as *Tch. tepidum* (LED) in this study.

2.2. Preparation of membranes

Chromatophores from *Tch. tepidum* and *Alc. vinosum* were prepared following the method described previously [30,31]. Chromatophores from *Rsp. rubrum*, *Rba. sphaeroides* and *Blc. viridis* were prepared by sonication (UD-200, 20 kHz, TOMY) of whole cells suspended in 50 mM Tris-HCl buffer (pH 8.5) followed by differential centrifugation at 4 °C and 150,000 × *g* for 90 min. To investigate the extent of solubilization by detergents on phospholipid composition of membranes, chromatophores from *Tch. tepidum* were treated with 0.2% (w/v) lauryldimethylamine *N*-oxide (LDAO, Kao) for different time periods. Unsolubilized fractions were collected as pellets by centrifugation at 4 °C and 150,000 × *g* for 90 min, and the supernatant containing the LH2 complex was dialyzed against distilled water and collected by centrifugation. The pellets were used for the subsequent extraction of phospholipids.

2.3. Preparation of LH1-RC complexes

Preparations of the LH1-RC complexes from *Tch. tepidum* and *Alc. vinosum* used similar methods to those described elsewhere [28,32,33] with minor modifications. In the last step of purification, the crude LH1-RC solutions were loaded onto a DEAE column (Toyopearl 650S, TOSOH) equilibrated with 20 mM Tris-HCl (pH 7.5) containing 0.05% (w/v) *n*-dodecyl-β-D-maltopyranoside (DDM, Anatrace) at 4 °C. The LH1-RC components were eluted by a linear gradient (0–50 mM) of $CaCl_2$. The LH1-RC complex from *Rsp. rubrum* was prepared using a previously described procedure [34]. For purification of the core complex from *Rba. sphaeroides*, the chromatophores were first treated with 0.4% (w/v) LDAO in 20 mM Tris-HCl buffer (pH 8.0) at 25 °C for 60 min to remove excess LH2, followed by centrifugation at 4 °C and 150,000 × *g* for 90 min. The pellets were then resuspended in the same buffer and extracted with 1.0% (w/v) OG (Anatrace) at 25 °C for 60 min followed by centrifugation at 4 °C and 150,000 × *g* for 90 min. The extracts were loaded onto the DEAE column (Toyopearl 650S)

equilibrated with 20 mM Tris-HCl (pH 8.0) containing 0.05% (w/v) DDM at 4 °C. Fractions of the LH1-RC were eluted by a linear gradient (0–400 mM) of NaCl. The LH1-RC complex from *Blc. viridis* was extracted from the chromatophores with 1.0% (w/v) DDM in 20 mM Tris-HCl buffer (pH 8.0) at 25 °C for 60 min followed by centrifugation at 4 °C and 150,000 ×*g* for 90 min. The extracts were loaded onto the DEAE column (Toyopearl 650S) equilibrated with 20 mM Tris-HCl (pH 8.0) containing 0.1% (w/v) DDM at 4 °C. Fractions of the LH1-RC were eluted by a linear gradient (0–400 mM) of NaCl. Freshly purified LH1-RC complexes in the eluted solutions were used directly for determining total phosphorous contents. Portions of the purified LH1-RC complexes were precipitated by addition of 40% (w/v) polyethylene glycerol 4000 and the pellets were used for phospholipid extractions.

2.4. Extraction of the phospholipids

Phospholipids were extracted using the Bligh and Dyer method [35]. Typically, two grams of wet pellets of the chromatophores were treated with a mixture of chloroform (5 mL)/methanol (10 mL)/water (MilliQ, 4 mL) for 10 min followed by centrifugation at 12,000 rpm for 10 min at 4 °C. The supernatant was mixed with 5 mL of chloroform and 4 mL of water and then vortexed for 5 min followed by centrifugation at 4,000 rpm for 10 min at 4 °C. The organic phase at the bottom was collected and dried by a vacuum pump. The extracts were washed with 10 mL of acetone and centrifuged at 12,000 rpm for 10 min at 4 °C. The precipitants containing extracted phospholipids were dried under an argon stream. A similar procedure was used for lipid extraction from LH1-RC complexes. The pellets of LH1-RC were first suspended in 2 mL of water (MilliQ) to adjust the suspension to an OD = 120 at the LH1 Q_y peak and then mixed with 5 mL of methanol and 2.5 mL of chloroform followed by centrifugation at 12,000 rpm for 10 min at 4 °C. The supernatant was mixed with 2.5 mL of chloroform and 3 mL of water, vortexed for 5 min, and then centrifuged at 4,000 rpm for 10 min at 4 °C. The precipitants were dried under an argon stream.

2.5. ^{31}P NMR measurements

Phospholipid compositions were analyzed by ^{31}P NMR spectroscopy according to the protocol reported elsewhere [36]. Briefly, the dried powders of extracted phospholipids were redissolved in 500 μL of chloroform-*d* and mixed with 200 μL of methanol and 50 μL of 0.2 M K-EDTA solution. The EDTA solution was prepared by titration of 0.2 M EDTA (free acid) with KOH to a pH of 6. ^{31}P NMR spectra were recorded at room temperature on a Bruker Biospin Avance III 500 MHz spectrometer equipped with a 5-mm broadband probe tuned to the ^{31}P nucleus frequency at 202.46 MHz. For quantitative analysis, the ^{31}P NMR spectra were acquired using inverse-gated proton decoupling (500.13 MHz) with the following parameters: 12175 Hz sweep width, 30° pulse, 8 K data points, 0.34-s acquisition time, 1.0-s delay time and 2000 scans. ^{31}P chemical shifts were referred to the peak of 85% H_3PO_4. Calibration of the ^{31}P NMR signals was carried out using a standard phospholipids kit (Doosan Serdary Research Laboratories, Canada) containing 10 phospholipids. The phospholipid compositions were calculated by integrating the ^{31}P NMR peak areas.

2.6. Inductively coupled plasma atomic emission spectroscopy (ICP-AES)

Total phosphorous contents were measured on a Shimadzu ICP-AES sequential plasma spectrometer (ICPS-7510). The purified LH1-RC fractions eluted from the DEAE column were adjusted to concentrations of OD = 5–10 at LH1 Q_y peaks. The detection wavelength was set to 213.620 nm. Multi-element Standard Solution W-I (Wako Pure Chemical) containing 1000 mg/L of P in H_2O was diluted to appropriate concentrations and used for calibration. Concentrations of the LH1-RC complexes were calculated from the LH1 Q_y intensities using the molar extinction coefficients of 4320 $mM^{-1}\,cm^{-1}$ for *Tch. tepidum* [37], 3800 $mM^{-1}\,cm^{-1}$ for *Rsp. rubrum* [34] and 3900 $mM^{-1}\,cm^{-1}$ for *Rba. sphaeroides* [38]. ICP-AES measurements were also employed in combination with absorption data to evaluate molar extinction coefficients of the LH1-RC complexes for *Alc. vinosum* (3800 $mM^{-1}\,cm^{-1}$) and *Blc. viridis* (2910 $mM^{-1}\,cm^{-1}$) according to the same procedure reported previously [39].

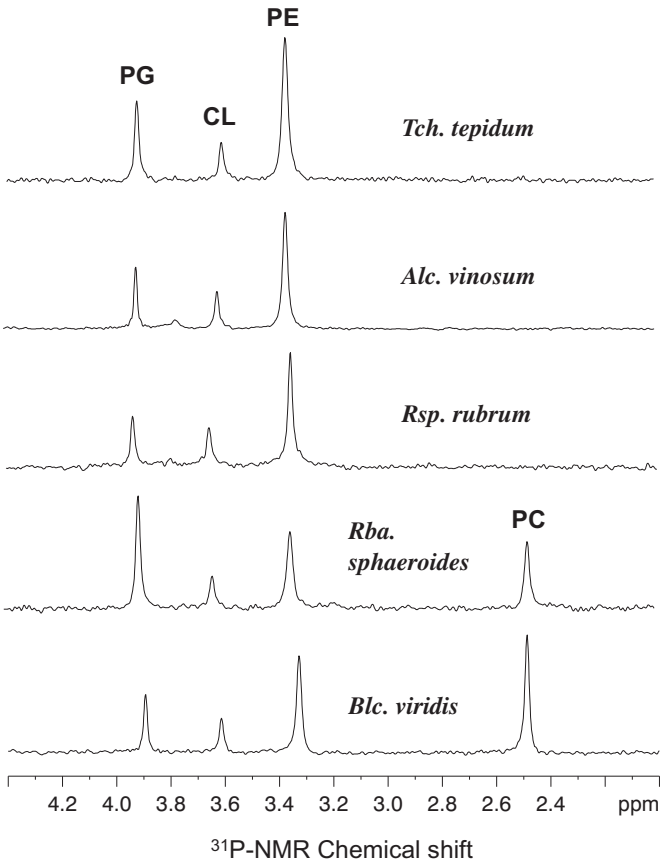

Fig. 1. ^{31}P NMR spectra of the phospholipids extracted from chromatophores of five purple phototrophic bacteria grown under incandescent illumination.

3. Results

In order to confirm the effectiveness of ^{31}P NMR spectroscopy for evaluation of the phospholipid composition, we conducted a reference experiment on *Escherichia coli* strain BL21 (Supporting information Fig. S1). Composition of the phospholipids were determined to be PE (73 mol%), PG (20 mol%) and CL (3.1 mol%). These values are in good agreement with those reported previously using a different method [40].

3.1. Phospholipid compositions in chromatophores

Fig. 1 shows ^{31}P NMR spectra of the phospholipids extracted from chromatophores of five purple bacteria, and Table 1 shows the compositions of phospholipids obtained. The signals for different phospholipids were well resolved. PE is the major phospholipid in the chromatophores of *Tch. tepidum* and *Alc. vinosum* followed by PG and CL. No PC was detected from these sulfur bacteria. The phospholipid composition of *Tch. tepidum* chromatophores from LED-illuminated cells were similar to those from cells grown with incandescent lighting (Supporting information Fig. S2), indicating that illumination wavelengths have little effect on phospholipid composition if light intensities are similar. Different light intensities were reported to result in slightly different phospholipid compositions in the cell membranes of *Rbl.*

Table 1
Distributions of the phospholipids in chromatophore membranes and purified LH1-RC complexes determined by ^{31}P NMR spectroscopy (mol %).

	PG	CL	PE	PC	Location
Tch. tepidum					
Incandescent	24 ± 3	10 ± 2	66 ± 5	nd[a]	Membrane
	11 ± 2	66 ± 4	23 ± 2	nd	LH1-RC
LED	18 ± 2	14 ± 1	68 ± 4	nd	Membrane
	20 ± 2	45 ± 3	35 ± 3	nd	LH1-RC
Alc. vinosum	25 ± 2	9 ± 1	66 ± 4	nd	Membrane
	28 ± 2	60 ± 4	12 ± 2	nd	LH1-RC
Rsp. rubrum	21 ± 2	12 ± 1	67 ± 4	nd	Membrane
	17 ± 2	44 ± 3	39 ± 3	nd	LH1-RC
Rba. sphaeroides	36 ± 2	6 ± 1	33 ± 3	25 ± 2	Membrane
	35 ± 3	55 ± 4	nd	10 ± 2	LH1-RC
Blc. viridis	21 ± 2	5 ± 1	31 ± 3	43 ± 3	Membrane
	9 ± 1	47 ± 4	16 ± 2	28 ± 3	LH1-RC

[a] nd, not detected.

acidophilus, with higher CL and PC but lower PG and PE under high light [23]. *Rsp. rubrum* chromatophores revealed a similar composition to that of the purple sulfur bacteria, and no PC was detected from this species, confirming previous qualitative analyses [4]. The results with *Rsp. rubrum* are somewhat different from those reported for *Rsp. rubrum* (strain NCIB 8255) previously, in which higher PG (38% w/w) and lower PE (38% w/w) proportions, together with a small amount of PC (5% w/w), were detected using a different method [41]. For *Rba. sphaeroides*, PG, PE and PC are the major phospholipids, whereas CL is a minor component. The composition is compatible with that reported for the same species using a similar method [29], but slightly different from those using different methods [7,8,42]. In *Blc. viridis*, PC and PE are the major components, accounting for more than 70% of the total phospholipids. Our results (Table 1) are qualitatively consistent with those reported previously for the same species [43], but differences exist for individual phospholipids, as the study by Linscheid et al. [43] reported 65 mol% PC, 11 mol% PE, 14 mol% PG and 10 mol% CL. The discrepancy may be interpreted in terms of the biosynthesis pathway of phospholipids [6], in which the PC and CL are biosynthesized from PE and PG, respectively. Because the sums of PC and PE (also the sums of PG and CL) are nearly identical between our results and those of Linscheid et al. [43], the differences observed in the compositions of individual phospholipids may reflect slight differences in the enzyme activities in the last steps of PC and CL biosynthesis or differences in growth conditions.

3.2. Changes of the phospholipid composition during solubilization

Many purple bacteria, including the species *Tch. tepidum*, *Alc. vinosum*, and *Rba. sphaeroides* used in this study, contain large amounts of LH2 in addition to LH1-RC, and LDAO is known to be an efficient detergent for selectively solubilizing the LH2 complex. We thus investigated the effect of LDAO solubilization on the phospholipid composition of *Tch. tepidum* membranes. Fig. 2 shows the ^{31}P NMR spectra of solubilized LH2 complex and the unsolubilized fractions at different extraction yields using 0.2% (w/v) LDAO. The extraction yields were measured as ratios of the LH2-B850 peaks of the extracts to that of chromatophores. Up to 70% extraction yields, the supernatant after centrifugation contained essentially LH2 complex as the sole pigment protein as judged from absorption spectrum (Supporting information Fig. S3). The LH2-rich fraction exhibited a phospholipid composition of more than 70 mol% PE and 13–14 mol% of PG and CL. The result is comparable with that obtained for a purified LH2 complex from *Rbl. acidophilus* [23] where 43 wt% PE, 42 wt% PC and 15 wt% CL were determined, but differs somewhat from those of *Rba. sphaeroides* type strain 2.4.1 where PG was identified as the major phospholipid in LH2 [29]. Since PC is absent from *Tch. tepidum* (Table 1), the proportion of

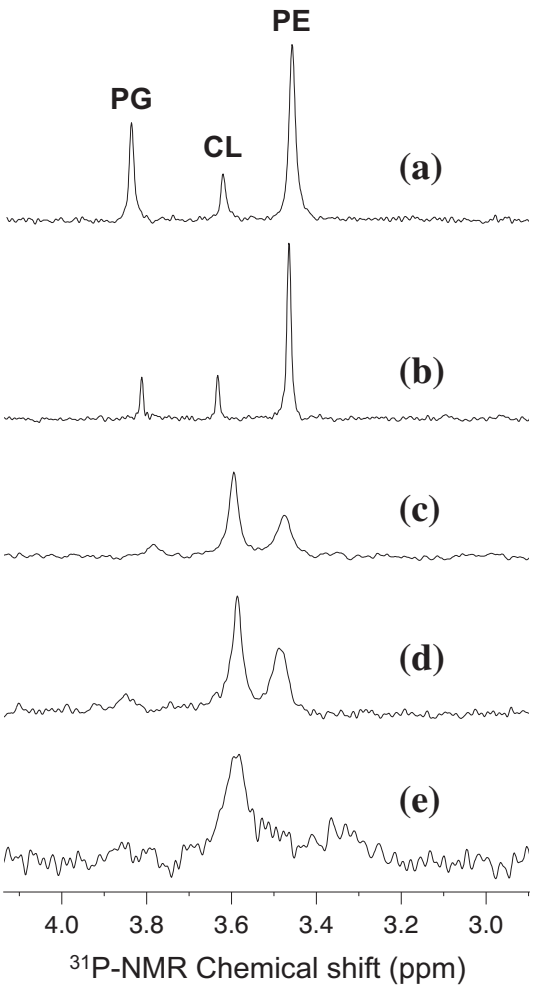

Fig. 2. Changes in ^{31}P NMR spectra of the phospholipid compositions during solubilization of the *Tch. tepidum* chromatophores using 0.2% (w/v) LDAO. (a) Chromatophores. (b) Solubilized LH2. (c–e) Unsolubilized pellets with extraction yields of 25% (c), 35% (d) and 40% (e).

the major component PE in the LH2-rich sample obtained in the present study is close to that of the sum of PE and PC in purified *Rbl. acidophilus* LH2. The unsolubilized *Tch. tepidum* fractions were obtained as pellets after centrifugation and contained a mixture of LH2 and LH1-RC complexes (a typical example is given in Supporting information Fig. S3). As the extraction yield increased from 25% to 40%, the proportions of PE decreased in the unsolubilized fractions along with an increase of CL from 35 mol% to more than 80 mol% (Fig. 2), and CL became the major phospholipid beyond an extraction yield of 40%. The unsolubilized fractions were used in the subsequent extraction of LH1-RC complex with other detergents in our work.

3.3. Phospholipid compositions in the purified LH1-RC complexes

All of the LH1-RC complexes were isolated to high purities as shown in Supporting information Fig. S4. Fig. 3 shows the ^{31}P NMR spectra of purified LH1-RC complexes from *Tch. tepidum*, and the quantified phospholipid compositions are given in Table 1. Upon purification, the minor component CL in photosynthetic membranes became the most abundant phospholipid in the core complexes from both incandescent and LED light grown cells, indicating that CL is preferentially associated with the LH1-RC complex. The proportion of CL was slightly higher in the complex from incandescent light grown cells whereas complexes from LED grown cells had a relatively higher proportion of PE (Table 1). A similar trend of CL enrichment as the major phospholipid was also

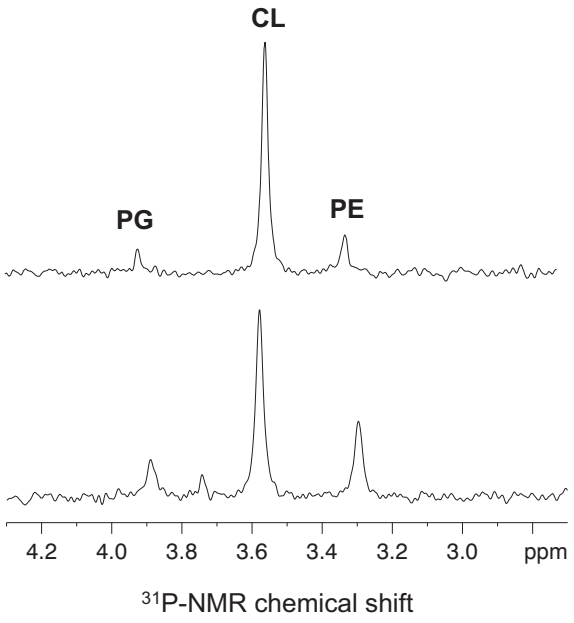

Fig. 3. ^{31}P NMR spectra of the phospholipids extracted from purified LH1-RC complexes of incandescent (top) and LED (bottom) light-grown *Tch. tepidum* cells.

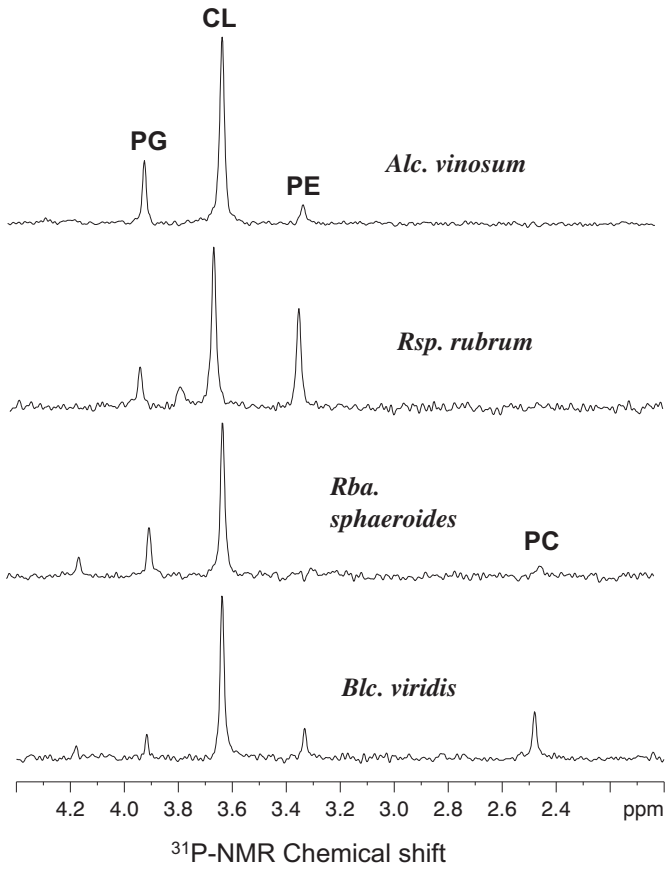

Fig. 4. ^{31}P NMR spectra of the phospholipids extracted from purified LH1-RC complexes of *Alc. vinosum*, *Rsp. rubrum*, *Rba. sphaeroides* and *Blc. viridis*.

observed for the LH1-RC complexes purified from other purple bacteria (Fig. 4 and Table 1). Interestingly, no PE was detected in the purified *Rba. sphaeroides* LH1-RC although PE was a major phospholipid in the membrane. In addition to the dominant CL, the PG content remained unchanged between chromatophores and LH1-RCs from *Alc. vinosum* and *Rba. sphaeroides* but decreased in the LH1-RCs from *Rsp. rubrum* and *Blc. viridis*. Moreover, the PE content of purified complexes from all species studied decreased significantly from their respective chromatophores as did the PC content of *Rba. sphaeroides* and *Blc. viridis* (Table 1). The results of *Rba. sphaeroides* LH1-RC in this study are consistent with that reported previously [29].

Table 2
Numbers of phospholipids per LH1-RC complex determined by ICP-AES and ^{31}P NMR spectroscopy.

	Total phosphorous	PG	CL	PE	PC
Tch. tepidum					
Incandescent	45 ± 5	3 ± 1	18 ± 2	6 ± 1	nd[a]
LED	45 ± 5	6 ± 1	14 ± 2	11 ± 2	nd
Crystals[b]		10	9	2	
Alc. vinosum	39 ± 4	7 ± 1	15 ± 2	3 ± 1	nd
Rsp. rubrum	33 ± 3	4 ± 1	10 ± 2	9 ± 2	nd
Rba. sphaeroides	48 ± 5	11 ± 2	17 ± 2	nd	3 ± 1
Blc. viridis	46 ± 5	3 ± 1	15 ± 2	5 ± 1	9 ± 2

[a] nd: not detected.
[b] From crystallographic structure (PDB: 5Y5S).

3.4. Number of phospholipids in the purified LH1-RC complexes

Total phosphorus contents in the purified LH1-RC complexes from various purple bacteria were determined by ICP-AES and the results are shown in Table 2. Under the conditions of the purification procedure used in this work, the numbers of phosphorus per LH1-RC for all bacteria fell into the range of 30–50. Different purification methods were reported to result in greater phosphorus contents for *Rba. sphaeroides* [29]. Combining the result of compositions determined by ^{31}P NMR spectra, the numbers of phospholipids in the purified LH1-RCs were calculated (Table 2). A total of 20–30 phospholipids were identified, which are thought to be tightly bound to the core complexes. Among these, 10–20 CL molecules were associated with each LH1-RC as the major phospholipid in all species. This indicates a specific interaction between the CL and the core complex. The remaining 10–15 phospholipids varied in the numbers of PG, PE and PC depending on the species. These numbers of the phospholipids are consistent with those confirmed in the recent high-resolution crystal structure of the LH1-RC from *Tch. tepidum* [28].

4. Discussion

^{31}P NMR and ICP-AES provide powerful tools for quantifying the phospholipid content of both chromatophores and purified photosynthetic complexes. However, determining the precise number of phospholipids associated with the LH1-RC complex faces at least two challenges: (1) large variations in the results obtained using different preparation methods, and (2) reproducibility of the results obtained even using the same procedure. Usually, different purification procedures and/or different detergents often yield different compositions and values for the number of phospholipids per core complex because the degree of replacement of the phospholipids by detergent molecules varies substantially between preparations. For example, it was demonstrated that the phosphorous contents for both dimeric and monomeric LH1-RC complexes from *Rba. sphaeroides*, purified by sucrose density gradient centrifugation with OG and sodium cholate, drastically reduced from 150 to 160 to 80–90 per RC upon detergent washing [29]. The latter was closer to the values of the phosphorous content determined in our study. These results indicate that there is a large fraction of the phospholipids that are only weakly bound to the core complexes and wash out as purification proceeds and that only tightly bound phospholipids are retained in the highly purified

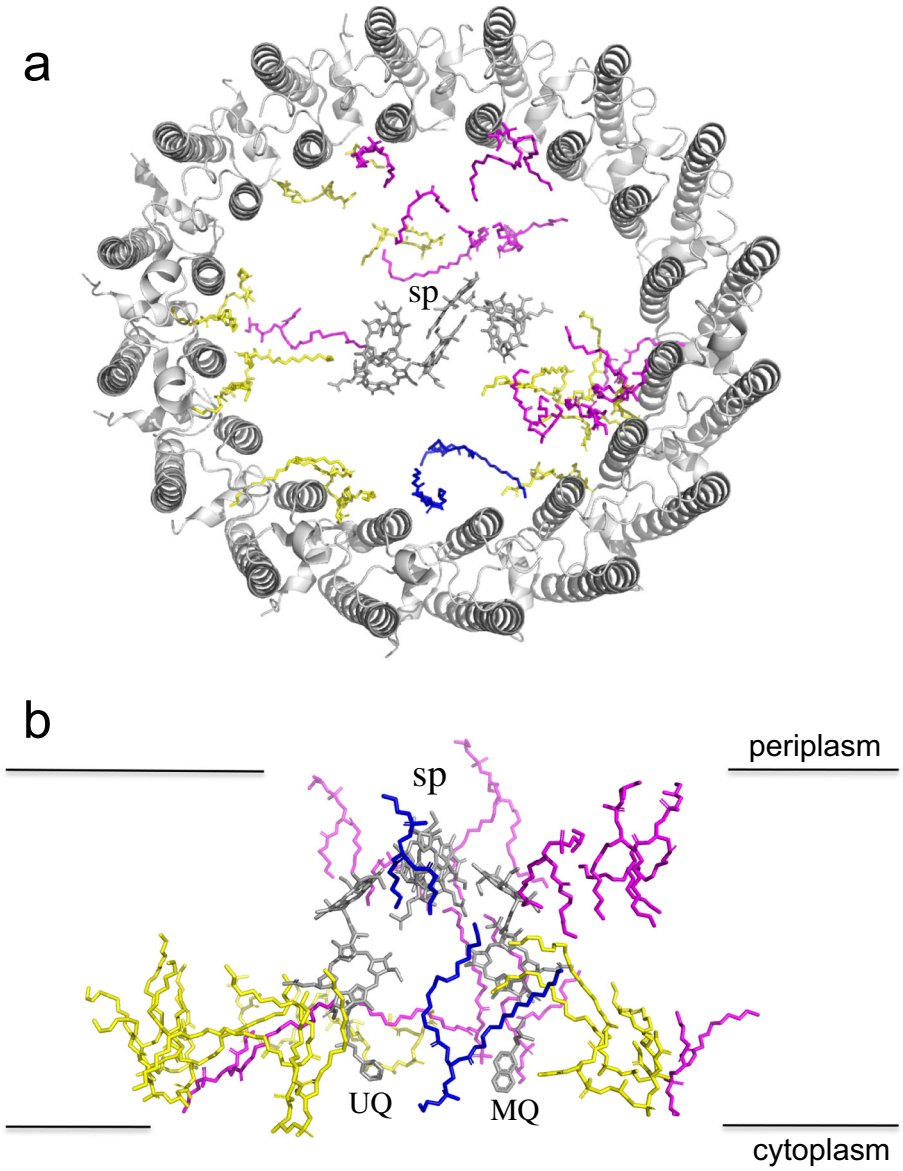

Fig. 5. Phospholipid distribution in the crystal structure of *Tch. tepidum* LH1-RC complex (PDB: 5Y5S). The pigment and quinone (UQ and MQ) molecules in RC (all side chains and the hemes in C-subunit were omitted for clarity) and the LH1 polypeptides are colored in grey. CL: yellow; PG: magenta; PE: blue. (a) Top view from the periplasmic side of membrane with the special pair (sp) positioned at the center. (b) Side view along the presumed membrane planes with the periplasm above and the cytoplasm below. The LH1 polypeptides were omitted for clarity.

complexes. Recently, SMA copolymers have been employed instead of surfactants for solubilizing photosynthetic membrane proteins [25–27]. This method exhibited a substantial difference in solubilization efficiencies for different proteins (high for RC and cytochrome bc_1 but low for LH1-RC), which was attributed to the size of the targeted protein and the density of packing of proteins in the membrane, and therefore reflects the local lipid environment of specific protein. Using the SMA, the core complex of *Rba. sphaeroides* was extracted as LH1-RC arrays rather than individual proteins and showed slightly increased CL in the phospholipid composition relative to that in the membranes [26].

The LH1-RC complexes purified from both incandescent and LED light-grown *Tch. tepidum* cells were used in our crystallization work, and the crystals consistently yielded high-resolution diffractions. Therefore, the results of the present study can be partly assessed by the crystallographic structures. It should be noted that disordered and/or low-occupancy phospholipids (even tightly bound) may not be resolved, or only partially resolved, in the structures even at high resolutions. When the crystal structure of *Tch. tepidum* LH1-RC was first solved at 3.0 Å resolution [44], two PG and one PE (only head groups) were provisionally modeled into the structure. Recently, the resolution was improved to 1.9 Å [28], and this allowed us to identify up to 21 phospholipids inside the core complex, which is within the range of the number determined in this work. However, if we consider that the complete assignments of phospholipids are still difficult at this resolution due to the weak electron density for some of the lipid molecules, as well as possible experimental errors in the quantification of the phospholipids, the lipid molecules assigned in the current structure represents most of the phospholipids in the LH1-RC complex.

In the present structure of the *Tch. tepidum* LH1-RC complex (PDB: 5Y5S), nine CL, ten PG and two PE molecules were assigned based on their electron density maps. One of the two PGs and the PE in the 3.0-Å structure (PDB: 3WMM) were reassigned as PE(PEF418) and CL (CDL304) in the 1.9-Å structure, respectively. All of the phospholipids in the new structure are located within the LH1 ring and fill the space between LH1 and the RC, suggesting a role in facilitating interactions between the two complexes. Based on the high-resolution structure, all

of the CL molecules were orientated toward the cytoplasmic side of the membrane with their head groups aligned toward the membrane surface, whereas the PG and PE molecules were distributed on both cytoplasmic and periplasmic sides as shown in Fig. 5. Most of the phospholipids were found to interact with the LH1 inner ring, and the head groups of almost all CL molecules interact with the basic residues, Arg18 and Arg19 in the LH1 α-polypeptide. The PG molecules on the periplasmic side make interactions mainly with Asn45 and Asp48 of the LH1 α-polypeptide. Several phospholipids identified in the *Tch. tepidum* LH1-RC structure correspond to the lipids resolved in the crystal structure of *Rba. sphaeroides* RC complex (PDB: 1M3X) [14]. For example, the PE(PEF418) occupies about half of the moiety of the CL in *Rba. sphaeroides* RC, two phospholipids (CDL104 and PGV414) are partially overlapped with the PC, and half of a CL(CDL304) occupies the position of the GGDG with an opposite orientation. Regarding the tightly bound CL in *Rba. sphaeroides* RC, careful inspection revealed some residual densities around the corresponding PE(PEF418) in the *Tch. tepidum* 1.9-Å structure, but an unambiguous assignment is impossible at this stage. Further improvements in resolution and the electron density map are required before this can be accomplished. Recently, a cryo-EM structure of LH1-RC from *Blc. viridis* became available at 2.9 Å resolution [45]. No specific lipid molecules were reported in this core complex although the gap region between LH1 and RC appears to be filled mainly with lipids and detergents that gave rise to disordered densities.

The specific localization of certain phospholipids in the bacterial photosynthetic core complexes is important in terms of the special roles these molecules play in the primary processes of photosynthesis and mechanism of molecular recognition. Since the CL can be viewed as a dimer of PG and both CL and PG have similar head groups with negative charges, they may have similar functions through electrostatic interactions with surrounding basic residues of proteins. In the present work, we showed that the minor component CL in the membrane becomes the major phospholipid in purified LH1-RC complex and that the sum of CL and PG accounts for more than two thirds of the total phospholipids present in the core complexes of a range of purple bacterial species. Kinetic studies on the detergent-solubilized LH1-RC and RC-only complexes from *Rba. sphaeroides* suggested that the charge separated state in the RC is stabilized by the CL molecules enriched in the complexes and this could be attributed to the local electrostatic interaction near the Q_A site by the anionic phospholipid [11,13,29]. By contrast, charge recombination experiments on the RCs purified with SMA copolymers showed stability exceeding that of the RCs isolated by detergents, with lifetimes close to that of the native chromatophore membrane [25]. The authors argued that while it is clear that CL is important for the charge separated state, the complete lipid complement appeared necessary to make the RCs retain the properties of the protein in its native environment.

A similar conclusion on the role of PG was also obtained from a study using the RC-only complex, where the PG was shown to significantly increase the lifetime of the $P^+Q_B^-$ state and the free energy gap that drives the Q_A^- to Q_B electron transfer [12]. PG is the only phospholipid found in the cyanobacteria and thylakoid membranes of chloroplasts, and is also significantly enriched in purified photosystem II complexes [46]. For the cyanobacterium *Thermosynechococcus vulcanus*, of the total of eight PG molecules per RC determined by biochemical analysis, five were identified in the crystal structure of photosystem II [47]. All of the PG molecules identified are located around the Q_A and Q_B sites. Deprivation of the PG and mutagenesis on the PG binding sites were shown to result in decreased photosynthetic activity presumably by inhibition of electron transport from Q_A to Q_B and destabilization of the binding of extrinsic proteins to the photosystem II [48–50]. Together with the results from our work, it seems that the preferential binding of the negatively charged lipids (including the anionic glycolipid sulfoquinovosyldiacylglycerol) to the RCs from bacteria to higher plants may have its roots in ancient phototrophic organisms and reflect a conserved function required for maintaining photosynthetic activities under drastically changing environments during the evolution.

Transparency document

The Transparency document associated with this article can be found, in online version.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dummy.2019.01.002.

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