



Microbial sources of intact polar diacylglycerolipids in the Western North Atlantic Ocean

Kimberly J. Popendorf^a, Michael W. Lomas^b, Benjamin A.S. Van Mooy^{a,*}

^a Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

^b Bermuda Institute of Ocean Sciences, St. George, Bermuda

ARTICLE INFO

Article history:

Received 22 December 2010

Received in revised form 11 April 2011

Accepted 7 May 2011

Available online 18 May 2011

ABSTRACT

Intact polar membrane lipids are essential components of microbial membranes and recent work has uncovered a diversity of them occurring in the ocean. While it has long been understood that lipid composition varies across microbial groups, the microbial origins of the intact polar lipids in the surface ocean remain to be fully explained. This study focused on identifying the microbial sources of intact polar diacylglycerolipids (IP-DAGs) in the surface waters of the western North Atlantic Ocean. We used three approaches to define these microbial sources: (i) ¹³C tracing to identify photoautotrophic and heterotrophic production of the major classes of IP-DAGs, (ii) cell sorting flow cytometry of *Prochlorococcus*, *Synechococcus* and heterotrophic bacteria to determine IP-DAG composition and (iii) regrowth incubations targeting IP-DAG production by heterotrophic bacteria. Stable isotope tracing indicated that sulfoquinovosyldiacylglycerol (SQDG) and diacylglyceryl-trimethyl-homoserine (DGTS) were produced predominantly by photoautotrophs, while phosphatidylglycerol (PG) production was dominated by heterotrophic bacteria. Of the cells sorted with flow cytometry, *Prochlorococcus* and *Synechococcus* were found to have abundant glycolipids, while heterotrophic bacteria were dominated by phospholipids. The regrowth incubations showed that the growth of heterotrophic bacteria correlated with an increase in the concentration of PG, phosphatidylethanolamine (PE) and monoglycosyldiacylglycerol (MGDG). The finding of MGDG in heterotrophic bacteria differs from previous work, which had asserted that the membranes of heterotrophic bacteria in this environment were composed entirely of phospholipids. Overall, our findings indicate that phytoplankton are the primary source of SQDG and DGTS, while heterotrophic bacteria are the dominant source of PG, making these three compounds promising biomarkers for the study of microbes in the surface ocean.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Membrane lipids are essential structural components of microbial cells. The particular mix of lipids forming the membrane defines its role in the microbial cell by affecting membrane fluidity, diffusive permeability and interaction with proteins (e.g. Zhang and Rock, 2008), among other properties. In the surface ocean, lipids comprise a substantial portion of cellular biomass (11–23% of the organic carbon in plankton; Wakeham et al., 1997) and cellular nutrient requirements (1–28% of plankton phosphate needs; Van Mooy et al., 2006). Production of membrane lipids is obligate for both growth and replication of cells, so membrane lipids are a significant cellular investment of resources and play a key role in the cycling of carbon and nutrients in the ocean.

Intact membrane lipids are attractive compounds for environmental microbial studies due to their structural diversity, which can be readily assessed using high performance liquid

chromatography/mass spectrometry analysis (HPLC–MS; Rütters et al., 2002; Sturt et al., 2004; Ertefai et al., 2008; Schubotz et al., 2009). The structures, defined by the head group and fatty acid moiety composition, can provide information on both the phylogenetic source of the lipids and the environmental conditions under which they were produced (Schubotz et al., 2009; Van Mooy and Fredricks, 2010). In addition, synthesis of membrane lipids is fundamental for cell growth, so their production rate has the potential to reflect microbial production (White et al., 1977; Van Mooy et al., 2008). Our study aimed to identify the microbial sources of intact membrane lipids in the surface ocean in order to increase their utility as molecular tools for studying the role of microbes in biogeochemical cycles.

Microbial membranes contain diverse classes of intact polar lipids, including phospholipids, glycolipids and betaine lipids (Kates, 1964; White and Tucker, 1969; Oliver and Colwell, 1973; King et al., 1977; Kato et al., 1996). With the advancement of HPLC–MS methodology it has been demonstrated that many representatives of these classes of microbial lipids are present in the surface ocean (Van Mooy et al., 2006, 2009; Schubotz et al., 2009; Van Mooy and

* Corresponding author. Tel.: +1 508 289 2322; fax: +1 508 457 2164.

E-mail address: bvanmooy@whoi.edu (B.A.S. Van Mooy).

Fredricks, 2010). Membranes of bacterial and eukaryotic plankton in the surface ocean are dominated by intact polar diacylglycerolipids (IP-DAGs). Deeper in the water column, the concentration of archaeal polar lipids with alkyl chains can be much more significant (Schubotz et al., 2009). This study focuses on the cohort of IP-DAGs common in open ocean environments (Van Mooy et al., 2009; Van Mooy and Fredricks, 2010), which includes (Fig. 1): (i) three classes of glycolipids – monoglycosyldiacylglycerol (MGDG), diglycosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG), (ii) three classes of phospholipids – phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) and (iii) three classes of betaine lipids – diacylglyceryl trimethylhomoserine (DGTS), diacylglyceryl hydroxymethyl-trimethyl- β -alanine (DGTA) and diacylglyceryl carboxyhydroxymethylcholine (DGCC).

Association between IP-DAG head groups and microbial groups has been established, such as that of the glycolipids MGDG, DGDG and SQDG with the thylakoid membranes of cyanobacteria and other phytoplankton (Wada and Murata, 1998; Sakurai et al., 2006) and that of betaine lipids with eukaryotic plankton (Vogel and Eichenberger, 1992; Kato et al., 1996; Guschina and Harwood, 2006; Van Mooy et al., 2009). These studies focused mainly on cultures, or demonstrated the co-occurrence of lipids and microbes in the ocean (e.g. Van Mooy and Fredricks, 2010). However, most marine microbes have not been cultured and little work has been carried out to directly link IP-DAGs with specific microbial groups in the surface ocean.

We have examined the microbial sources of membrane lipids from environmental samples using three distinct approaches: (i) ^{13}C -labeled substrates to trace lipid production by photoautotrophs and heterotrophic bacteria, (ii) cell sorting flow cytometry to separate cyanobacteria and heterotrophic bacteria for IP-DAG analysis and (iii) regrowth incubations to examine the production of IP-DAGs during growth of heterotrophic bacteria. Our results indicate that several classes of IP-DAGs have the potential to be useful biomarkers for studying microbial processes in the sea.

2. Methods

2.1. Cruises and sampling scheme

Samples were collected on two cruises in the North Atlantic (Fig. 2): (i) the BV39 cruise in October 2007 aboard the R/V Atlantic Explorer, which was a meridional transect from Bermuda to Puerto Rico and (ii) the Oc443 cruise in April 2008 aboard the R/V Oceanus, which comprised a leg from Woods Hole, Massachusetts northeast to $43^\circ\text{N } 65^\circ\text{W}$ and a second leg south along 65°W towards Bermuda. Samples were collected throughout both cruises for determination of soluble reactive phosphate and cell abundance. At five stations on the BV39 cruise and three stations on the Oc443 cruise, samples were collected for cell sorting flow cytometry. In the southern, oligotrophic portion of the Oc443 cruise water was collected for stable isotope incubations and in the northern portion of the BV39 cruise water for regrowth incubation. Water for all samples was collected using Niskin bottles mounted on a rosette equipped with conductivity, temperature and pressure sensors.

2.2. Phosphate concentration

For both cruises, samples were collected at multiple depths at each station or cast for determination of soluble reactive phosphorus (SRP). Seawater samples (ca. 200 ml) were collected directly from the Niskin bottles into high density polypropylene bottles and frozen at -20°C until analysis. SRP was measured using the MAGIC method (Karl and Tien, 1992).

2.3. Stable isotope incubations

2.3.1. Experimental design

The study used incubations of whole seawater with ^{13}C -labeled substrates to identify IP-DAGs produced by autotrophs, organisms

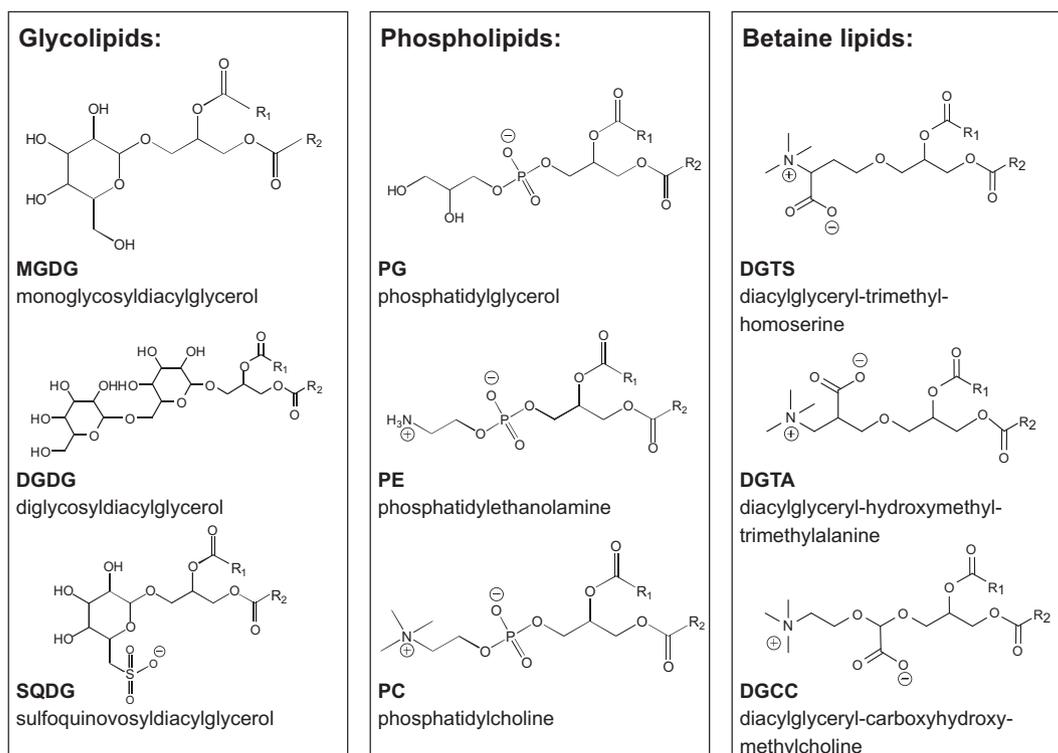


Fig. 1. IP-DAGs dominating plankton membranes in the surface ocean (R_1 and R_2 represent FA moieties).

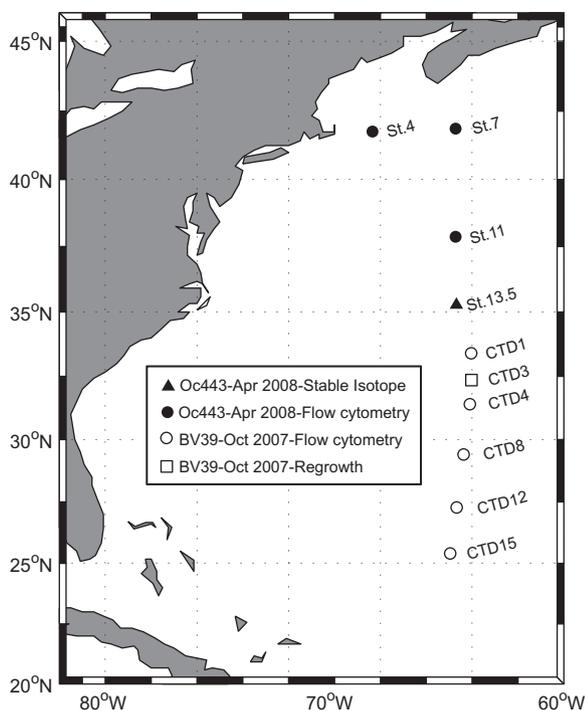


Fig. 2. Sampling locations during two cruises in the North Atlantic Ocean.

that use inorganic carbon as carbon source (here ^{13}C -labeled bicarbonate) and heterotrophs, organisms that use organic carbon as their carbon source (here ^{13}C -labeled glucose). Incubations were conducted in the light and the dark, with the ^{13}C -bicarbonate incubation in the light (denoted as ^{13}C -bicarbonate light), targeting IP-DAG production by photoautotrophs, and the ^{13}C -glucose incubation in the dark (^{13}C -glucose dark) the most targeted for production by heterotrophic bacteria. Incubations were conducted aboard the R/V Oceanus during the Oc443 cruise in April 2008. Water was collected at Station 13.5 at 35.5°N, 65.0°W (Fig. 2). Seawater from 20 m was collected using Niskin bottles and transferred immediately to three acid-washed clear polycarbonate carboys using acid-washed Tygon tubing. There were three conditions for the three carboys: (i) addition of ^{13}C -labeled glucose to a final concentration of ca. 100 nM ($2\text{-}^{13}\text{C}$ D-glucose; Cambridge Isotope Laboratories, Inc.), (ii) addition of ^{13}C -labeled bicarbonate to a final concentration of ca. 20 μM (Cambridge Isotope Laboratories, Inc.) and (iii) a control with no substrate addition. Immediately following addition of ^{13}C -labeled substrate, the carboys were placed in on-deck flow through incubators at surface seawater temperature. Incubation in the dark used 20 l carboys and an opaque incubator, while the experiment in the light used 10 l carboys and a clear plexiglass incubator covered with neutral density screen which blocked ca. 50% of the surface irradiance. All incubations lasted 24 h. At the end of incubation the entire contents of the carboys were filtered onto 0.2 μm Anodisc filters (Whatman) using vacuum filtration (ca. -200 mm Hg), ca. 1 l per filter. Filters were wrapped in combusted Al foil and stored in liquid N_2 until extraction.

2.3.2. Lipid extraction and analysis

Lipids were extracted from the filters using a modified Bligh and Dyer protocol (Bligh and Dyer, 1959), as described by Van Mooy and Fredricks (2010). Phosphatidylethanolamine-N-(2,4-dinitrophenyl) (DNP-PE; Avanti Polar Lipids) was added with the initial solvent as an internal recovery standard. Total extracts were analyzed using HPLC–MS with a Hewlett Packard 1100 HPLC instrument and Thermo-Finnigan LCQ Deca XP ion trap mass

spectrometer with electrospray ionization (ESI) interface. Full HPLC and MS methods are provided by Van Mooy and Fredricks (2010), as updated from Sturt et al. (2004). Eight IP-DAG classes were identified: MGDG, SQDG, PG, and PE were identified by characteristic neutral fragment loss in positive ion mode, while PC, DGTS, DGTA and DGCC were identified by characteristic fragment ions in positive ion mode (Sturt et al., 2004; Van Mooy and Fredricks, 2010). Once molecular ions had been established for each IP-DAG class, the eight classes were purified using preparative HPLC with an Agilent 1200 HPLC instrument equipped with a UV/visible variable wavelength detector, a fraction collector, and an Agilent LC/MSD SL mass spectrometer. Retention times were established by way of MSD detection in positive ion full scan mode of the previously assigned molecular ion of each IP-DAG, and these were used to set conservative time-based fraction collection windows for each IP-DAG (Fig. 3).

2.3.3. Fatty acid (FA) processing and analysis

Each IP-DAG fraction was transesterified to produce FA methyl esters (FAMES). The intact lipids were reacted (ca. 2 h) with methanolic HCl under N_2 at 70 °C. FAMES were analyzed using gas chromatography isotope ratio mass spectrometry (GC-IRMS) to determine relative abundance and $\delta^{13}\text{C}$ values (‰) relative to Pee Dee Belemnite. FAMES were identified by comparison of retention times with a standard (Sigma–Aldrich, Supelco 37-component FAME Mix 47885-U). Samples for GC-IRMS (gas chromatography-isotope ratio mass spectrometry) were injected from hexane/ CH_2Cl_2 via a programmable temperature vaporizing inlet (Gerstel PTV CIS-4), operated in solvent venting mode, to a Hewlett Packard 6890 GC instrument with Varian CP-Sil 5 CB LB column (60 m \times 0.25 mm id \times 0.25 μm phase) with a 1 m guard column and He flow of 1 ml/min. Combustion was with a Finnigan-MAT GC Combustion III interface coupled to a DeltaPlus stable isotope ratio mass spectrometer, where the interface had been modified to an integral fused silica design (Goodman, 1998). Data were acquired and manipulated with the Finnigan-MAT IsodatNT software package. To compare the enrichment of the IP-DAGs under the different incubation conditions, the average enrichment of each purified IP-DAG was expressed as the weighted average enrichment of the composite FAs for that sample. This was calculated as the sum of the enrichment of each FA multiplied by its abundance, divided by the total abundance of FAs. The control incubations with no ^{13}C -labeled substrate were used to determine an average $\delta^{13}\text{C}$ value of -27.6‰ (st. dev. 1.0‰) for FAs with no isotopic

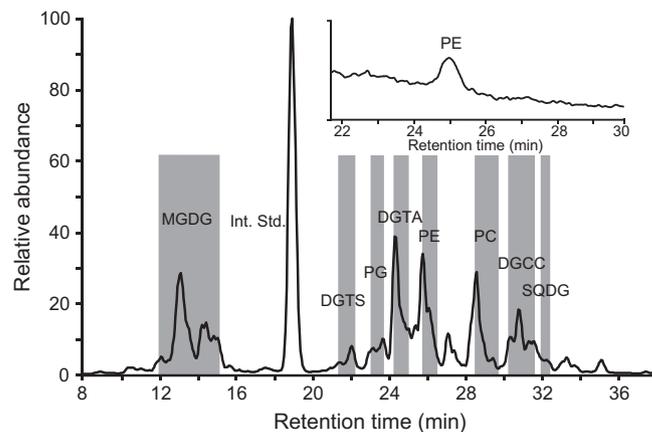


Fig. 3. Base peak ion chromatogram of a representative total lipid extract from the stable isotope incubations showing major IP-DAG classes. Grey bars indicate time-based fraction collection windows for each IP-DAG class. Inset is an example total ion chromatogram for a PE fraction after separation, showing the purity of the fractions after collection.

enrichment and all other $\delta^{13}\text{C}$ values are given as enrichment relative to this baseline.

2.4. Cell sorting flow cytometry

2.4.1. Sampling and flow cytometry

Samples for cell sorting flow cytometry were collected at three locations on the Oc443 cruise in April 2008 and at five locations on the BV39 cruise in October 2007; at each location three to six depths were sampled in the upper 120 m of the water column. Each sample (1–2 l) was collected in Niskin bottles and transferred to an acid-washed polycarbonate bottle before filtration. Samples were filtered using vacuum filtration (ca. –200 mm Hg) onto 47 mm diameter 0.2 μm polycarbonate filters (Whatman) for BV39 samples or 47 mm diameter 0.2 μm hydrophilic Durapore filters (Millipore) for Oc443 samples. Filtration was stopped when ca. 1 ml liquid remained on the filter to avoid directly exposing live unfixed cells to air; the remaining volume and filter were gently transferred to 5 ml cryovials, diluted to 5 ml with 0.2 μm filtered low nutrient seawater and fixed with paraformaldehyde to a concentration of 1%. Samples were fixed (15 min) at room temperature and then at 4 °C (several h) before being flash frozen in liquid N_2 . They were kept frozen in liquid N_2 until being thawed for flow cytometry sorting at the Bermuda Institute of Ocean Sciences. Cyanobacteria were sorted according to methods given by Casey et al. (2009). Heterotrophic bacteria were sorted as follows: 1 ml aliquots of homogenized sample were transferred to 5 ml polypropylene Falcon tubes (BD Biosciences, San Jose, CA). Cell suspension was stained (10 min) with 1 μl nucleic acid dye SYTO-13 (0.2 μm filter-sterilized; 5 mM in dimethyl sulfoxide; Invitrogen, Carlsbad, CA) at room temperature in the dark. After laser noise and bubble exclusion with FSC pulse height and width, panels and gating hierarchy were adapted from Guindulain et al. (1997) to exclude *Prochlorococcus* and *Synechococcus* from heterotrophic bacteria by SSC (pulse height), FITC (pulse area 530/40 nm), PE (pulse height 580/30 nm) and “Chlorophyll” (pulse height 692/40 nm) fluorescence. Cells were sorted at 27.5 PSI and rate was maintained below 20,000 s^{-1} . Recovery and purity for heterotrophic bacterial sorts were 96 \pm 3% and 98 \pm 6%, respectively ($n = 7$). Mean abort rates were consistently <5%. Sorted cells were deposited into 5 ml polypropylene Falcon tubes, flash frozen in liquid N_2 and stored at –80 °C until extraction.

2.4.2. Extraction and analysis

Thawed samples were combined before extraction as follows: Oc443 samples from each station (St. 4, St. 7 and St. 11) all depths were combined; for BV39 samples, all depths from CTD4 were combined, and for casts CTD1, CTD8, CTD12 and CTD15 samples from the same depth were combined to give samples from 5 m, 20 m, 40 m, and the deep chlorophyll maximum (80 m, 115 m, 120 m and 110 m at the respective casts; see Supplementary material Table 1). After combining samples, lipids were extracted using a modified Bligh and Dyer extraction protocol as above with the following adjustment: for samples BV39-CTD4 and BV39-40 m, the total liquid volume of the cell sorts was used in place of phosphate buffer; for all other samples the cell sorted samples were combined and vacuum filtered (ca. –200 mm Hg) onto 47 mm 0.02 μm Anodisc filters (Whatman) and the filters extracted as for stable isotope samples. HPLC–MS analysis of the extract used an Agilent 1200 HPLC instrument coupled to a Thermo Scientific TSQ Vantage triple quadrupole mass spectrometer with heated ESI interface using HPLC conditions as in Section 2.3.2. IP-DAG classes were quantified in positive ion mode by integrating the total ion current of either the constant neutral loss scan, parent ion scan, or selected reaction monitoring (SRM) scan as follows: MGDG neutral loss of 197 da; SQDG neutral loss of 261 da; DGDG neutral loss

of 359 da; PG neutral loss of 189 da; PE neutral loss of 141 da; PC product ion of m/z 184; DNP-PE (internal standard) SRM for the transition of m/z 875 to m/z 551. Response factors for each IP-DAG were established by analyzing standard curves prepared with synthetic PG, PE, PC, and DNP-PE (Avanti Polar Lipids Inc., Alabaster, AL), natural MGDG and DGDG standards (Matreya, LLC, Pleasant Gap, PA) and natural SQDG (Lipid Products, South Nutfield, UK). One sample of *Prochlorococcus* (BV39-CTD4) had an anomalously high concentration of PC, most likely due to low specificity of cell sorting at this location, possibly because of high numbers of conjoint *Prochlorococcus* and heterotrophic bacteria (Malfatti and Azam, 2009). It was not included in calculations of average IP-DAG composition of *Prochlorococcus*.

2.5. Regrowth incubation

2.5.1. Experimental design and analysis

Incubations were conducted on board the R/V Atlantic Explorer during the BV39 cruise in October 2007. Seawater was collected at 32.610°N 64.100°W at 5 m depth and transferred from Niskin bottles to acid-washed 20 l polycarbonate carboys using acid-washed Tygon tubing. Triplicate carboys were prepared with 2 l whole seawater and 18 l seawater gravity-filtered through an acid-cleaned 0.2 μm Polycap 36 TC filter (hydrophilic polyethersulfone membrane, Whatman). Carboys were incubated (72 h) in a dark temperature controlled room (within 2 °C of surface water temperature) and sampled every 12 h for cell counts and IP-DAG concentration. For IP-DAG concentration, 1 l of water was filtered onto a 47 mm 0.2 μm Anodisc filter (Whatman) using vacuum filtration (ca. –200 mm Hg). Filters were wrapped in Al foil and stored in liquid N_2 . For flow cytometry cell counts, 1.5 ml water was added to a 2.0 ml cryovial and fixed with paraformaldehyde for a final concentration of 1%. Samples were stored at –80 °C until analysis. Extraction and analysis of glycolipids and phospholipids were conducted as above for the flow cytometry sorted samples. Betaine lipids were not analyzed.

3. Results

3.1. Phosphate concentration

On the Oc443 cruise, Stations 4 and 7 were north of the Gulf Stream and phosphate concentration ranged from 350 nM to 500 nM in the upper 100 m (Supplementary material, Table 1), while Stations 11 and 13.5 were south of the Gulf Stream where phosphate concentration in the upper 100 m was consistently <40 nM. The BV39 transect, from Bermuda to Puerto Rico, was entirely in the oligotrophic waters of the Sargasso Sea, and phosphate concentration in the upper 100 m was consistently <20 nM and often <10 nM.

3.2. Planktonic sources of IP-DAGs probed via stable isotope tracing

In the ^{13}C -bicarbonate light incubation, each IP-DAG class showed substantial isotopic enrichment above the controls, except for PG. The most enriched lipid in the ^{13}C -bicarbonate light incubation was MGDG, with a weighted average composite FA enrichment of 188‰ above the controls (Fig. 4). The average enrichment of SQDG was 52‰, the second highest enrichment in the ^{13}C -bicarbonate light incubation. The other IP-DAG classes – PE, PC, DGTA and DGCC – showed enrichment ranging from 20‰ to 40‰ in the ^{13}C -bicarbonate light incubation. The enrichment of DGTS was comparatively low at 15‰. In the ^{13}C -bicarbonate dark incubation, none of the IP-DAGs showed substantial enrichment.

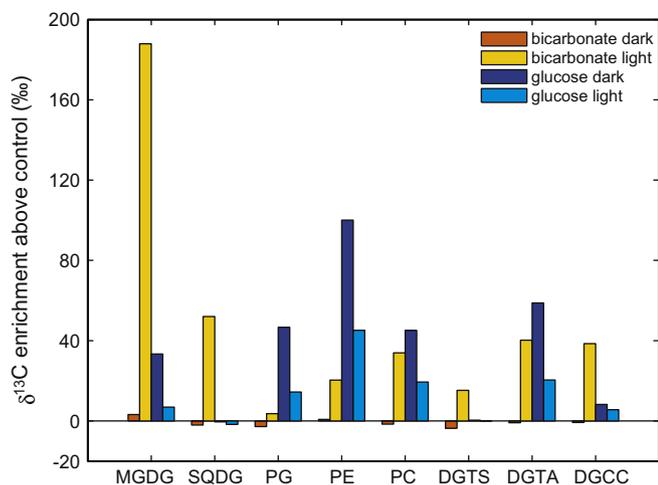


Fig. 4. Average isotopic enrichment of eight IP-DAGs from whole seawater incubated with ^{13}C -labeled substrates under light and dark conditions. Data are presented as enrichment above 27.6‰, the average $\delta^{13}\text{C}$ of the control samples with no isotope addition.

In the ^{13}C -glucose dark incubation, most IP-DAG classes showed substantial ^{13}C enrichment, with the exception of SQDG and DGTS (Fig. 4). The most enriched IP-DAG class was PE at 100‰, while enrichment of PG was 47‰. The other four IP-DAGs enriched in the ^{13}C -glucose incubations (MGDG, PC, DGTA and DGCC) had enrichment ranging from 8‰ to 59‰ in the ^{13}C -glucose dark incubation. For every IP-DAG class that showed enrichment in the ^{13}C -glucose dark incubation, it also showed enrichment in the ^{13}C -glucose light incubation but to a lesser extent. The six IP-DAGs isotopically enriched in the ^{13}C -glucose incubations were 2.8 ± 1.2 times more enriched in the dark incubation than in the light incubation.

In summary, three IP-DAGs showed ^{13}C enrichment exclusively with one substrate: SQDG and DGTS were enriched only in the ^{13}C -bicarbonate light incubation and PG was enriched only in the ^{13}C -glucose incubations. The other classes of IP-DAGs – MGDG, PE, PC, DGTA and DGCC – were enriched whether incubated with ^{13}C -bicarbonate or ^{13}C -glucose.

3.3. Planktonic sources of IP-DAGs probed by way of cell sorting flow cytometry

Samples were collected for cell sorting flow cytometry at two locations where phosphate concentration was high (Stations 4 and 7) and at six where phosphate concentration was low. Though *Prochlorococcus* was more abundant than *Synechococcus* at the stations with low phosphate, *Synechococcus* was present in a wider geographic range, extending to the nutrient-rich waters in the north. Heterotrophic bacteria were abundant throughout the transect, averaging 3.0×10^5 cells/ml, and showed no consistent correlation with geography or depth (Supplementary material, Table 1). Phospholipids and glycolipids were abundant in the extracts from the sorted cells, but betaine lipids were below the level of quantification and so were not included in the subsequent data analysis.

Five samples of *Prochlorococcus* were analyzed, all from the oligotrophic sampling sites, and on average 86% of the membrane lipids were glycolipids (Fig. 5). The most abundant IP-DAG was MGDG, averaging 50% of the membrane lipids, followed by SQDG (36%); the other glycolipid, DGDG, was <1%. Phospholipids comprised 14% of the membrane lipids on average; the most abundant was PG (9%), followed by PE (4%) and PC (<1%).

Seven samples of *Synechococcus* were analyzed; glycolipids composed an average of 82% of the membrane lipids and were

dominated by MGDG at 55%, followed by SQDG at 20% (Fig. 5). In contrast to *Prochlorococcus*, most of the *Synechococcus* samples contained the glycolipid DGDG, averaging 7% of the detected lipids. The phospholipid contribution to the membrane was roughly split between PG and PE (avg. 9% and 8% of the total membrane lipids) with PC contributing <1% (Fig. 5).

The membrane composition of the cyanobacteria (average of five samples of *Prochlorococcus* and seven of *Synechococcus*) averaged 16% phospholipids and 84% glycolipids for all locations (Fig. 6a). For *Synechococcus*, the difference in membrane composition between the samples from high and low phosphate stations was striking, the two samples from high phosphate locations having an average of 50% glycolipids (Fig. 6), and the five from low phosphate locations having an average of 94% glycolipids. Combining the data from all ten samples of cyanobacteria (*Prochlorococcus* and *Synechococcus*) for low phosphate locations, glycolipids comprised 90% of the membrane lipids (Fig. 6).

Over the eight samples of heterotrophic bacteria, the average membrane composition was 70% phospholipids and 30% glycolipids (Figs. 5 and 6). The phospholipids in the heterotrophic bacteria were roughly equally distributed between PG, PE, and PC (25%, 19% and 26%), while glycolipids were almost exclusively MGDG (27%). The membrane composition of heterotrophic bacteria was quite different between the high and low phosphate locations, with phospholipids averaging 97% of the membrane in the two samples from high phosphate locations and 62% in the six samples from low phosphate locations (Fig. 6).

In summary, the extracts from *Prochlorococcus*, *Synechococcus* and heterotrophic bacteria sorted by way of flow cytometry demonstrated broad differences in the membrane composition between the cyanobacteria and the heterotrophic bacteria, as well as differences in membrane composition between samples from locations with low or high phosphate concentration.

3.4. Heterotrophic bacterial sources of IP-DAGs probed by way of regrowth incubations

In order to directly examine the link between the growth of heterotrophic bacteria and the production of IP-DAGs, a “regrowth” experiment was set up with 90% filtered (0.2 μm) seawater and

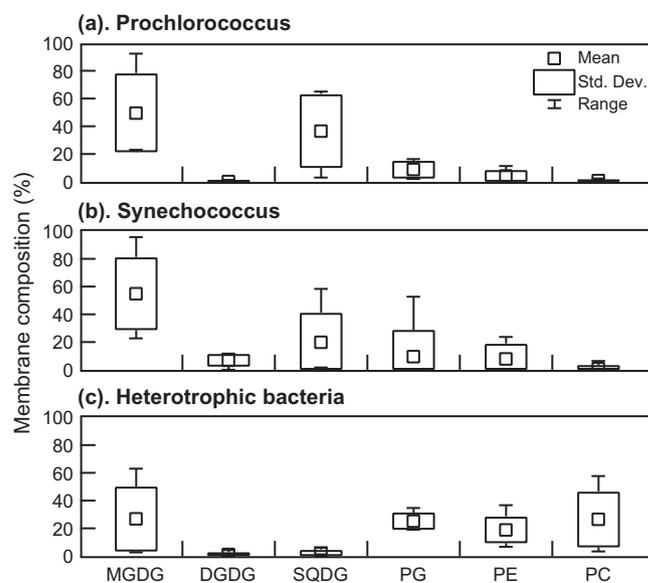


Fig. 5. Average IP-DAG content of *Prochlorococcus* (a), *Synechococcus* (b) and heterotrophic bacteria (c) from samples collected across the North Atlantic and sorted by way of flow cytometry prior to lipid extraction.

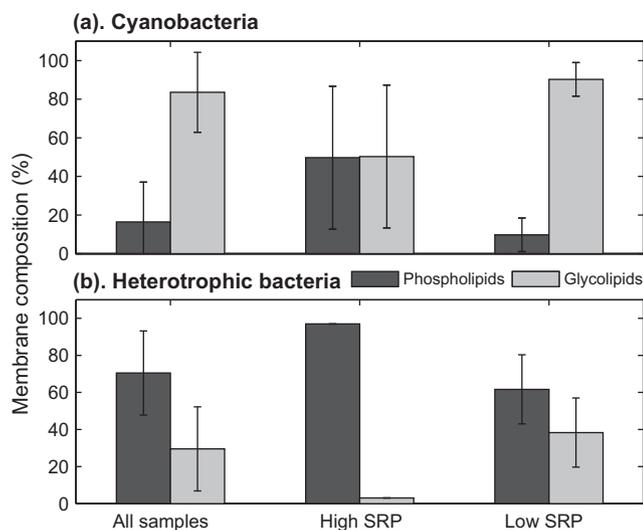


Fig. 6. Relative amounts of glycolipids and phospholipids in cyanobacteria (a) and heterotrophic bacteria (b), as an average of all samples ($n = 12$ for cyanobacteria, $n = 8$ for heterotrophic bacteria), locations with high SRP (>250 nM, $n = 2$, $n = 2$) and locations with low SRP (<40 nM, $n = 10$, $n = 6$). Error bars are \pm one standard deviation.

10% whole sea, which were incubated in the dark to target growth of heterotrophic bacteria. With reduced grazing pressure and unaltered ambient nutrients, the heterotrophic bacterial abundance increased from 9.0×10^3 cells/ml to 3.5×10^4 cells/ml over 3 days, while the abundance of *Prochlorococcus*, *Synechococcus* and eukaryotes remained very low and constant (Fig. 7). Concurrent analysis of IP-DAGs from the whole community (all cells >0.2 μm) showed that the abundance of PC, DGDG and SQDG also remained relatively constant, while MGDG, PG and PE increased (Fig. 7). Heterotrophic bacteria abundance was significantly positively correlated with MGDG, PG and PE concentration (R^2 0.45, 0.44 and 0.53 respectively, $p < 0.05$).

4. Discussion

Our goal was to determine the microbial sources of the most abundant classes of IP-DAGs in the North Atlantic. Any class of IP-DAG shown to be unique to a microbial group offers the promise of being applied in future studies as a biomarker for that group. However, it is important to note that the three approaches we applied, while complementary, yield information on the sources of IP-DAGs from operationally defined groups of plankton whose definitions vary according to the sampling method. In addition, the study spanned a range of locations. Thus some disagreement in the sources of IP-DAGs identified using the different approaches was expected. For example, the organisms that incorporated ^{13}C into IP-DAGs from ^{13}C -glucose were not necessarily identical to the heterotrophic bacteria isolated via cell sorting flow cytometry. Indeed, some phytoplankton species are known to take up glucose, while some heterotrophic bacteria cannot (Neilson and Lewin, 1974; Alonso-Sáez and Gasol, 2007; Schwabach et al., 2010). Similarly, the heterotrophic bacteria isolated via flow cytometry were not necessarily identical to those heterotrophic bacteria that grew in the regrowth incubations; it has been reported that the communities of heterotrophic bacteria that emerge in these types of incubations may differ from the initial inoculums (Fuchs et al., 2000; Carlson et al., 2002; Yokokawa et al., 2004). With these qualifications in mind, we drew on the data provided by these different approaches in order to form a consensus, where possible, on sources of classes of IP-DAGs.

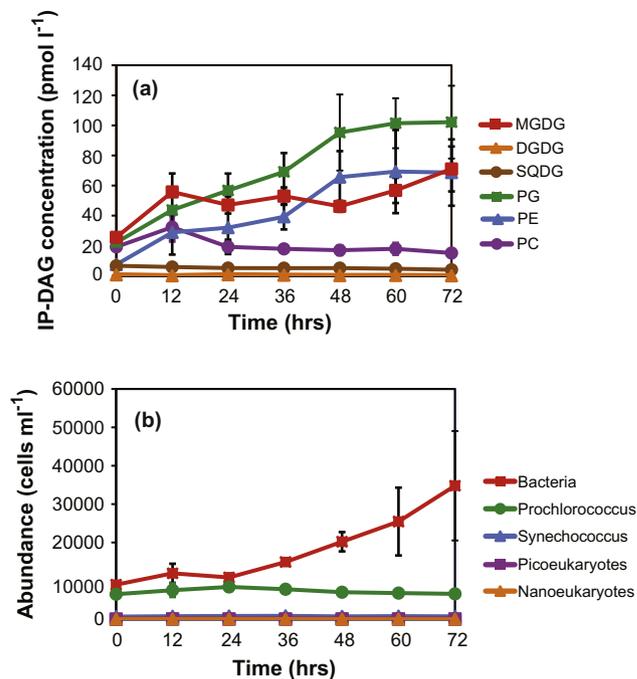


Fig. 7. IP-DAG concentration (a) and cell abundance (b) over a 72 h regrowth incubation. Error bars are standard deviation of single analyses from triplicate incubations.

4.1. Microbial sources of IP-DAGs

4.1.1. SQDG

The ^{13}C enrichment of SQDG from incubations amended with ^{13}C -bicarbonate in the light, along with the absence of ^{13}C enrichment in the ^{13}C -glucose incubations, suggests that this class of IP-DAG was produced primarily by photoautotrophs (Fig. 4). This is supported by the abundance of SQDG in cyanobacteria and lack of SQDG in heterotrophic bacteria collected via cell sorting flow cytometry (Fig. 5). Furthermore, SQDG was found not to increase with the abundance of heterotrophic bacteria in the regrowth incubations, as observed by Van Mooy et al. (2009). Previous studies have shown SQDG to be a significant fraction of the membrane lipids in cyanobacteria cultures (Benning, 1998; Wada and Murata, 1998; Van Mooy et al., 2006) and have identified a close association between SQDG abundance and cyanobacterial abundance in the ocean (Van Mooy and Fredricks, 2010). A survey of shotgun genomic sequences from the Sargasso Sea did not place gene sequences for SQDG synthesis (sqdB; Benning, 1998) in known heterotrophic clades (Van Mooy et al., 2006). Together, published work and our data support the conclusion that photoautotrophs are the nearly exclusive source of SQDG in the surface ocean.

4.1.2. DGTS

The betaine lipid DGTS was also isotopically enriched only in the ^{13}C -bicarbonate light incubation (Fig. 4), indicating that it too was produced exclusively by photoautotrophs. The ^{13}C enrichment of classes of IP-DAGs is indicative of the proportional rate at which the IP-DAG is turned over during biosynthesis (e.g. the quotient of the synthesis rate and the cellular concentration). The lower ^{13}C enrichment of DGTS than SQDG may be because DGTS turns over more slowly in the cell than SQDG as cellular maintenance repairs and replaces membrane lipids (White and Tucker, 1969; Zhang and Rock, 2008). Alternatively, DGTS could have been produced by a different set of photoautotrophs than SQDG and these organisms may have been turning over more slowly than those that dominate production of SQDG. The association of betaine lipids

with eukaryotic phytoplankton in previous studies (Kato et al., 1996; Van Mooy and Fredricks, 2010) supports the latter hypothesis, as eukaryotic phytoplankton likely have a lower growth rate than prokaryotic cyanobacteria (Furnas, 1990; Veldhuis et al., 1993). In addition, DGTS abundance was below our limit of quantification in both *Prochlorococcus* and *Synechococcus*. Betaine lipids were not measured in the regrowth incubation, but in a similar incubation conducted in the Sargasso Sea betaines were shown not to increase with increasing abundance of heterotrophic bacteria (Van Mooy et al., 2009). We therefore posit that eukaryotic phytoplankton are the primary source of DGTS in the surface ocean. Little is known of the functional role of DGTS in marine microbial cells, but the substantial difference in enrichment between DGTS and DGTA, with very similar structures (Fig. 1), indicates that these betaine lipids may be produced by distinct clades of phytoplankton. Indeed, few marine organisms are known to produce both compounds (Harwood, 1998; Van Mooy et al., 2006).

4.1.3. PG and PE

The phospholipid PG was substantially enriched only in the ^{13}C -glucose incubations (Fig. 4), indicating that it was probably produced primarily by heterotrophic bacteria. Importantly, it is the only IP-DAG that showed no appreciable enrichment in the ^{13}C -bicarbonate light incubation, precluding substantial synthesis of PG by photoautotrophs. In the heterotrophic bacteria isolated via flow cytometry, PG was a major lipid (Fig. 5), whereas in the cyanobacteria PG accounted for only a small fraction of the total IP-DAGs, particularly in low phosphate samples (Fig. 6); the average percentage of PG in cyanobacteria membranes was less than half that in heterotrophic bacteria membranes. This is consistent with previous work that established that, although PG is present in both heterotrophic bacteria (Oliver and Colwell, 1973; Van Mooy et al., 2006) and cyanobacteria (Wada and Murata, 1998; Sato et al., 2000a,b; Sato, 2004; Van Mooy et al., 2006), cyanobacteria contain little PG in environments where phosphate is scarce (Van Mooy et al., 2009). Given that heterotrophic bacteria generally outnumber cyanobacteria by an order of magnitude in the western North Atlantic (Cavender-Bares et al., 2001; Supplementary material, Table 1) we conclude that the vast majority of PG in the low phosphate waters of the Sargasso Sea originates from heterotrophic bacteria despite their somewhat smaller size. The increase in PG concentration, correlated with the increase in heterotrophic bacterial cells in the regrowth experiment (Fig. 7), lends further support to heterotrophic bacteria being the major source of PG.

Previous studies of cultures found that PE is absent from cyanobacteria membranes, but is abundant in heterotrophic bacteria membranes (Oliver and Colwell, 1973; Wada and Murata, 1998; Van Mooy et al., 2006). The membrane of *Pelagibacter ubique*, the most abundant heterotrophic bacterium in the Sargasso Sea, was shown to be composed solely of PE and PG (Van Mooy et al., 2009). Like these culture results, the flow cytometry sorted samples showed only minor amounts of PE in cyanobacteria and much more abundant PE in heterotrophic bacteria. Since PE has not been observed in any axenic culture of cyanobacteria (Wada and Murata, 1998), we suggest that the PE in the sorted *Prochlorococcus* and *Synechococcus* cells indicates that they might be contaminated with small amounts of heterotrophic bacteria, either due to nonspecific sorting inherent in our flow cytometry methods (sort purity ca. 98%; Section 2.4.1) or possibly due to misassignment of cells in the sorting process because of aggregation of cyanobacteria and heterotrophic bacteria (Malfatti and Azam, 2009). If so, this would also suggest that at least some of the PG in the sorted *Synechococcus* and *Prochlorococcus* could also have originated from heterotrophic bacteria.

The results of the 3 day regrowth incubation corroborate a heterotrophic bacterial source of PE: the increase in heterotrophic bacterial cells correlated with an increase in PE abundance

(Fig. 7). This, along with data from flow cytometry sorted cells (Fig. 5), suggests that production of PE may be dominated by heterotrophic bacteria. However, despite the relative lack of PE in cyanobacteria, PE was isotopically enriched in both the ^{13}C -glucose incubations and the ^{13}C -bicarbonate light incubations (Fig. 4), consistent with production by both heterotrophic bacteria and photoautotrophs. However, in comparing the results of the flow cytometry sorted samples and stable isotope incubations, it is important to note that the sorted *Prochlorococcus*, *Synechococcus* and heterotrophic bacteria cells do not represent the full microbial community in the environment. In contrast, the isotopic enrichments in the stable isotope incubations represent production by all the microbes ($>0.2\ \mu\text{m}$) in the environment, which includes pico- and nano-eukaryotes and larger organisms from which lipids were not sampled in the cell sorting flow cytometry. Therefore, the isotopic enrichment of PE in the ^{13}C -bicarbonate light incubation may represent production of PE by eukaryotic phytoplankton. Alternatively, it may represent secondary enrichment, whereby autotrophic organisms took up the ^{13}C -bicarbonate and produced ^{13}C -labeled dissolved organic carbon that was subsequently taken up by heterotrophic bacteria and incorporated into PE.

4.1.4. MGDG

In the ^{13}C -bicarbonate light incubation, MGDG was the most enriched class of IP-DAG. However, it was also enriched in the ^{13}C -glucose incubations, suggesting that it was produced by both photoautotrophs and heterotrophic bacteria. Indeed, the flow cytometry sorted samples demonstrated that MGDG was abundant in both cyanobacteria and heterotrophic bacteria. In the regrowth incubation MGDG abundance increased with increasing heterotrophic bacterial cells, reinforcing the idea that MGDG was a substantial component of heterotrophic bacteria membranes in the oligotrophic Sargasso Sea. These results differ from culture studies, mostly conducted with phosphate-replete media, which have found heterotrophic bacteria membranes to be exclusively phospholipids (Kates, 1964; Oliver and Colwell, 1973; Van Mooy et al., 2006, 2009).

4.1.5. Other IP-DAGs

In the flow cytometry sorted samples, PC was abundant in heterotrophic bacteria and not in cyanobacteria (Fig. 5), in agreement with previous culture studies (Goldfine, 1972; Wada and Murata, 1998; Van Mooy et al., 2006). However, the regrowth incubations suggested that PC was not a major component of heterotrophic bacteria (Fig. 7). Yet PC is also known to be present in eukaryotic phytoplankton (Kato et al., 1996; Guschina and Harwood, 2006; Van Mooy et al., 2009), so it was not surprising that the PC was enriched in incubations with both ^{13}C -bicarbonate and ^{13}C -glucose (Fig. 4). Thus, we reach the same conclusion as Van Mooy and Fredricks (2010): potential planktonic origins of PC in the surface ocean are diverse, although eukaryotic phytoplankton is likely to be a dominant source.

The glycolipid DGDG was scarce in the flow cytometry sorted samples and regrowth incubation, and its abundance was too low to be measured in the stable isotope incubations, indicating it makes a minimal contribution to the membranes of marine microbes. This is in accord with studies that have found low concentrations of DGDG in surface seawater relative to the other glycolipids and phospholipids (Van Mooy and Fredricks, 2010).

In the flow cytometry sorted samples, betaine lipid levels were below our level of quantification in the prokaryotic groups collected; previous work would suggest that betaine lipids are not abundant in either *Prochlorococcus*, *Synechococcus* or heterotrophic bacteria (Van Mooy et al., 2009; Van Mooy and Fredricks, 2010). However, betaine lipids have been shown to have similar concentrations to glycolipids in seawater (Van Mooy and Fredricks, 2010), and presumably originate from a eukaryotic source (Kato et al., 1996; Van Mooy et al., 2009). The enrichment in incubations with

both ^{13}C -glucose and ^{13}C -bicarbonate indicate that DGTA and DGCC were probably produced by both heterotrophic bacteria and autotrophs. Therefore, based on the available information, we conclude that these two betaine lipids are unlikely to originate from any single group of plankton.

4.2. Stable isotope enrichment for light vs. dark incubation

The ^{13}C -bicarbonate dark incubation showed no isotopic enrichment of any of the lipids (Fig. 4), despite recent findings that heterotrophic bacteria can be responsible for substantial uptake of bicarbonate in the dark under some conditions (Alonso-Sáez et al., 2010). Recently several studies have focused on the uptake of dissolved inorganic carbon in the deep sea (Jost et al., 2008; Hansman et al., 2009; Varela et al., 2011). However, these studies were conducted with seawater rich in reduced inorganic nutrients (e.g. NH_4^+ , H_2S), whereas our incubations were conducted with nutrient-depleted surface waters where highly oxidizing conditions prevail. If chemolithoautotrophs were present in our incubations, we suggest that other resident heterotrophic bacteria and phytoplankton would outcompete prokaryotic chemolithoautotrophs for nutrients, limiting their ability to take up bicarbonate in our dark incubations. Given the lack of ^{13}C enrichment of IP-DAGs in the ^{13}C -bicarbonate dark incubation, we can reasonably conclude that photoautotrophs were responsible for the observed uptake of ^{13}C -bicarbonate in our light incubation.

For every IP-DAG isotopically enriched in the ^{13}C -glucose incubations (MGDG, PG, PE, PC, DGTA, and DGCC), the ^{13}C enrichment in the dark incubation was roughly twofold more than in the light. There are two potential mechanisms to explain this pattern: (i) in the light, the photoautotrophs produced organic compounds that diluted the ^{13}C -labeled glucose in a broader pool of bioavailable organic carbon or (ii) in the light, the active photoautotrophs outcompeted the heterotrophic bacteria for nutrients (phosphate, NH_3 , etc.), which caused the heterotrophic bacteria to grow more slowly and incorporate less ^{13}C -glucose. Although this isotopic discrepancy is surprisingly uniform across all these classes of IP-DAGs, at this time we do not have evidence to support or refute either of these two mechanisms.

4.3. Influence of phosphate concentration on membrane composition

It has recently been shown that the ability to substitute non-phosphorus membrane lipids for phospholipids may provide an advantage to organisms in phosphorus-limited environments, and that this ability may be confined to specific microbial groups (Van Mooy et al., 2009). Cyanobacteria and eukaryotic plankton have been shown to be capable of lipid substitution (Benning et al., 1995; Van Mooy et al., 2009), while heterotrophic bacteria have been hypothesized to be incapable of substitution. It would therefore be expected that in low phosphate environments, such as the Sargasso Sea, cyanobacteria would produce non-phosphorus lipids, while heterotrophic bacteria would produce primarily phospholipids. While it was demonstrated here that heterotrophic bacteria almost certainly dominate the production of the phospholipids PG and PE, it was also shown that the fraction of phospholipids varied with phosphate concentration for both cyanobacteria and heterotrophic bacteria. The IP-DAGs of heterotrophic bacteria in the high phosphate samples were almost exclusively phospholipids, whereas in the low phosphate samples MGDG composed more than one third of the total measured lipids (Fig. 6). Furthermore, the regrowth incubations also suggested that heterotrophic bacteria were a source of MGDG (Fig. 7). These results might indicate that heterotrophic bacteria are capable of substituting MGDG for phospholipids in oligotrophic surface waters as a low phosphorus adaptive strategy in a fashion similar to the

substitution of SQDG for PG in cyanobacteria and betaine lipids for PC in eukaryotic plankton (Benning, 1998; Sato et al., 2000a,b; Van Mooy et al., 2009). In contrast to the other proposed lipid substitutions, the ionic charge of MGDG is not similar to the phospholipids it seems to be replacing; in the pH range of seawater, MGDG is neutral, while PG is anionic and PE and PC are zwitterionic. The ionic charge of IP-DAGs is thought to be important for maintaining membrane function. Whether or not a “substitution” mechanism is at work, it seems clear that MGDG can be a significant component of heterotrophic bacteria membranes in phosphorus-limited surface ocean environments, in contrast to previous work (Van Mooy et al., 2009; Van Mooy and Fredricks, 2010).

In high phosphate locations, north of the Gulf Stream, *Prochlorococcus* was not abundant enough for collection by cell-sorting flow cytometry, so the composition of cyanobacterial membranes in the high phosphate region could only be assessed for *Synechococcus*; this distribution of *Prochlorococcus* and *Synechococcus* is similar to previous observations (Cavender-Bares et al., 2001). The IP-DAGs of *Synechococcus* in the high phosphate locations were roughly half glycolipids and half phospholipids, while in the low phosphate locations the IP-DAGs were almost entirely glycolipids. In accord with the established sulfolipid substitution hypothesis (Benning, 1998; Yu et al., 2002; Van Mooy et al., 2006), the SQDG fraction of cyanobacterial membranes in the low phosphate samples was more than fivefold greater than in the high phosphate samples, reinforcing the conditions of phosphate limitation in the Sargasso Sea and the utility of IP-DAGs as indicators of the concomitant physiological response.

5. Conclusions

There are significant differences in the membrane composition between cyanobacteria and heterotrophic bacteria in the western North Atlantic. The membranes of cyanobacteria were predominantly glycolipids, while those of heterotrophic bacteria were predominantly, though not exclusively, phospholipids. Specifically, the production of the phospholipid PG, and potentially also PE, was dominated by heterotrophic bacteria, while production of the glycolipid SQDG was dominated by photoautotrophs. Production of the betaine lipid DGTS was also dominated by photoautotrophs, though apparently not *Prochlorococcus* or *Synechococcus*. These findings indicate that these classes of IP-DAG molecules could be important biomarkers for different groups of plankton. In particular, by more thoroughly constraining the dominant planktonic sources of IP-DAGs, our findings provide additional support for combining isotope tracing methods and IP-DAG analysis to determine growth rates of different groups of plankton in the upper ocean (e.g. Van Mooy et al., 2008).

Acknowledgments

We thank K. Longnecker and J. Ossolinski for contributions to the experiments conducted at sea. Likewise, we thank the crews of the R/V Oceanus and R/V Atlantic Explorer. We are also extremely grateful to J. Casey for diligence and dedication in the course of optimizing the cell-sorting flow cytometry methods. Finally, we thank C. Johnson and H. Fredricks for assistance with lipid analysis and two anonymous reviewers for helpful comments.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.orggeochem.2011.05.003.

References

- Alonso-Sáez, L., Gasol, J.M., 2007. Seasonal variations in the contributions of different bacterial groups to the uptake of low-molecular-weight compounds in Northwestern Mediterranean Coastal Waters. *Applied and Environmental Microbiology* 73, 3528–3535.
- Alonso-Sáez, L., Galand, P.E., Casamayor, E.O., Pedrós-Alió, C., 2010. High bicarbonate assimilation in the dark by Arctic bacteria. *The ISME Journal* 4, 1581–1590.
- Benning, C., 1998. Biosynthesis and function of the sulfolipid sulfoquinovosyl diacylglycerol. *Annual Review of Plant Physiology and Plant Molecular Biology* 49, 53–75.
- Benning, C., Huang, Z.-H., Gage, D.A., 1995. Accumulation of a novel glycolipid and a betaine lipid in cells of *Rhodobacter sphaeroides* grown under phosphate limitation. *Archives of Biochemistry and Biophysics* 317, 103–111.
- Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Physiology and Pharmacology* 37, 911–917.
- Carlson, C.A., Giovannoni, S.J., Hansell, D.A., Goldberg, S.J., Parsons, R., Otero, M.P., Vergin, K., Wheeler, B.R., 2002. Effect of nutrient amendments on bacterioplankton production, community structure, and DOC utilization in the northwestern Sargasso Sea. *Aquatic Microbial Ecology* 30, 19–36.
- Casey, J.R., Lomas, M.W., Michelou, V.K., Dyhrman, S.T., Orchard, E.D., Ammerman, J.W., Sylvan, J.B., 2009. Phytoplankton taxon-specific orthophosphate (Pi) and ATP utilization in the western subtropical North Atlantic. *Aquatic Microbial Ecology* 58, 31–44.
- Cavender-Bares, K.K., Karl, D.M., Chisholm, S.W., 2001. Nutrient gradients in the western North Atlantic Ocean: relationship to microbial community structure and comparison to patterns in the Pacific Ocean. *Deep-Sea Research I* 48, 2373–2395.
- Ertefai, T.F., Fisher, M.C., Fredricks, H.F., Lipp, J.S., Pearson, A., Birgel, D., Udert, K.M., Cavanaugh, C.M., Gschwend, P.M., Hinrichs, K.-U., 2008. Vertical distribution of microbial lipids and functional genes in chemically distinct layers of a highly polluted meromictic lake. *Organic Geochemistry* 39, 1572–1588.
- Fuchs, B.M., Zubkov, M.V., Sahn, K., Burkill, P.H., Amann, R., 2000. Changes in community composition during dilution cultures of marine bacterioplankton as assessed by flow cytometric and molecular biological techniques. *Environmental Microbiology* 2, 191–201.
- Furnas, M.J., 1990. In situ growth rates of marine phytoplankton: approaches to measurement, community and species growth rates. *Journal of Plankton Research* 12, 1117–1151.
- Goldfine, H., 1972. Comparative aspects of bacterial lipids. *Advances in Microbial Physiology* 8, 1–58.
- Goodman, K., 1998. Hardware modifications to an isotope ratio mass spectrometer continuous-flow interface yielding improved signal, resolution, and maintenance. *Analytical Chemistry* 70, 833–837.
- Guindulain, T., Comas, J., Vives-Rego, J., 1997. Use of nucleic acid dyes SYTO-13, TOTO-1, and YOYO-1 in the study of *Escherichia coli* and marine prokaryotic populations by flow cytometry. *Applied and Environmental Microbiology* 63, 4608–4611.
- Guschina, I.A., Harwood, J.L., 2006. Lipids and lipid metabolism in eukaryotic algae. *Progress in Lipid Research* 45, 160–186.
- Hansman, R.L., Griffin, S., Watson, J.T., Druffel, E.R.M., Ingalls, A.E., Pearson, A., Aluwihare, L., 2009. The radiocarbon signature of microorganisms in the mesopelagic ocean. *Proceedings of the National Academy of Sciences USA* 106, 6513–6518.
- Harwood, J.L., 1998. Membrane lipids in algae. In: Siegenthaler, P.-A., Murata, N. (Eds.), *Lipids in Photosynthesis: Structure, Function and Genetics*. Kluwer Academic Publishers, Netherlands, pp. 53–64.
- Jost, G., Zubkov, M.V., Yakushev, E., Labrenz, M., Jürgens, K., 2008. High abundance and dark CO₂ fixation of chemolithoautotrophic prokaryotes in anoxic waters of the Baltic Sea. *Limnology and Oceanography* 53, 14–22.
- Karl, D.M., Tien, G., 1992. MAGIC: a sensitive and precise method for measuring dissolved phosphorus in aquatic environments. *Limnology and Oceanography* 37, 105–116.
- Kates, M., 1964. Bacterial lipids. *Advances in Lipid Research* 2, 17–90.
- Kato, M., Sakai, M., Adachi, K., Ikemoto, H., Sano, H., 1996. Distribution of betaine lipids in marine algae. *Phytochemistry* 42, 1341–1345.
- King, J.D., White, D.C., Taylor, C.W., 1977. Use of lipid composition and metabolism to examine structure and activity of estuarine detrital microflora. *Applied and Environmental Microbiology* 33, 1177–1183.
- Malfatti, F., Azam, F., 2009. Atomic force microscopy reveals microscale networks and possible symbioses among pelagic marine bacteria. *Aquatic Microbial Ecology* 58, 1–14.
- Neilson, A.H., Lewin, R.A., 1974. The uptake and utilization of organic carbon by algae: an essay in comparative biochemistry. *Phycologia* 13, 227–264.
- Oliver, J.D., Colwell, R.R., 1973. Extractable lipids of gram-negative marine bacteria: phospholipid composition. *Journal of Bacteriology* 114, 897–908.
- Rütters, H., Sass, H., Cypionka, H., Rullkötter, J., 2002. Phospholipid analysis as a tool to study complex microbial communities in marine sediments. *Journal of Microbiological Methods* 48, 149–160.
- Sakurai, I., Shen, J.-R., Leng, J., Ohashi, S., Kobayashi, M., Wada, H., 2006. Lipids in oxygen-evolving photosystem II complexes of cyanobacteria and higher plants. *Journal of Biochemistry* 140, 201–209.
- Sato, N., 2004. Roles of the acidic lipids sulfoquinovosyl diacylglycerol and phosphatidylglycerol in photosynthesis: their specificity and evolution. *Journal of Plant Research* 117, 495–505.
- Sato, N., Hagio, M., Wada, H., Tsuzuki, M., 2000a. Environmental effects on acidic lipids of thylakoid membranes. In: Harwood, J.L., Quinn, P.J. (Eds.), *Recent Advances in the Biochemistry of Plant Lipids*. Portland Press, London, pp. 912–914.
- Sato, N., Hagio, M., Wada, H., Tsuzuki, M., 2000b. Requirement of phosphatidylglycerol for photosynthetic function in thylakoid membranes. *Proceedings of the National Academy of Sciences USA* 97, 10655–10660.
- Schubotz, F., Wakeham, S.G., Lipp, J.S., Fredricks, H.F., Hinrichs, K.-U., 2009. Detection of microbial biomass by intact polar membrane lipid analysis in the water column and surface sediments of the Black Sea. *Environmental Microbiology* 11, 2720–2734.
- Schwalbach, M.S., Tripp, H.J., Steindler, L., Smith, D.P., Giovannoni, S.J., 2010. The presence of the glycolysis operon in SAR11 genomes is positively correlated with ocean productivity. *Environmental Microbiology* 12, 490–500.
- Sturt, H.F., Summons, R.E., Smith, K., Elvert, M., Hinrichs, K.-U., 2004. Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry – new biomarkers for biogeochemistry and microbial ecology. *Rapid Communications in Mass Spectrometry* 18, 617–628.
- Van Mooy, B.A.S., Rocap, G., Fredricks, H.F., Evans, C.T., Devol, A.H., 2006. Sulfolipids dramatically decrease phosphorus demand by picocyanobacteria in oligotrophic environments. *Proceedings of the National Academy of Sciences USA* 103, 8607–8612.
- Van Mooy, B.A.S., Moutin, T., Duhamel, S., Rimmelin, P., Van Wambeke, F., 2008. Phospholipid synthesis rates in the eastern subtropical South Pacific Ocean. *Biogeosciences* 5, 133–139.
- Van Mooy, B.A.S., Fredricks, H.F., Pedler, B.E., Dyhrman, S.T., Karl, D.M., Koblížek, M., Lomas, M.W., Mincer, T.J., Moore, L.R., Moutin, T., Rappé, M.S., Webb, E.A., 2009. Phytoplankton in the ocean substitute phospholipids in response to phosphorus scarcity. *Nature* 458, 69–72.
- Van Mooy, B.A.S., Fredricks, H.F., 2010. Bacterial and eukaryotic intact polar lipids in the eastern subtropical South Pacific: water-column distribution, planktonic sources, and fatty acid composition. *Geochimica Cosmochimica Acta* 74, 6499–6516.
- Varela, M.M., van Aken, H.M., Sintès, E., Reinthaler, T., Herndl, G.J., 2011. Contribution of *Crenarchaeota* and *Bacteria* to autotrophy in the North Atlantic interior. *Environmental Microbiology*. doi:10.1111/j.1462-2920.2011.02457.x.
- Veldhuis, M.J.W., Kraay, G.W., Gieskes, W.W.C., 1993. Growth and fluorescence characteristics of ultraplankton on a north-south transect in the eastern North Atlantic. *Deep-Sea Research II* 40, 609–626.
- Vogel, G., Eichenberger, W., 1992. Betaine lipids in lower plants. Biosynthesis of DGTS and DGTA in *Ochromonas danica* (Chrysothymaceae) and the possible role of DGTS in lipid metabolism. *Plant Cell Physiology* 33, 427–436.
- Wada, H., Murata, N., 1998. Membrane lipids in cyanobacteria. In: Siegenthaler, P.-A., Murata, N. (Eds.), *Lipids in Photosynthesis: Structure, Function and Genetics*. Kluwer Academic Publishers, Netherlands, pp. 65–81.
- Wakeham, S.G., Hedges, J.I., Lee, C., Peterson, M.L., Hernes, P.J., 1997. Compositions and transport of lipid biomarkers through the water column surficial sediments of the equatorial Pacific Ocean. *Deep-Sea Research II* 44, 2131–2162.
- White, D.C., Tucker, A.N., 1969. Phospholipid metabolism during bacterial growth. *Journal of Lipid Research* 10, 220–233.
- White, D.C., Bobbie, R.J., Morrison, S.J., Oosterhof, D.K., Taylor, C.W., Meeter, D.A., 1977. Determination of microbial activity of estuarine detritus by relative rates of lipid biosynthesis. *Limnology and Oceanography* 22, 1089–1099.
- Yokokawa, T., Nagata, T., Cottrell, M.T., Kirchman, D.L., 2004. Growth rate of the major phylogenetic bacterial groups in the Delaware estuary. *Limnology and Oceanography* 49, 1620–1629.
- Yu, B., Xu, C., Benning, C., 2002. Arabidopsis disrupted in SQD2 encoding sulfolipid synthase is impaired in phosphate-limited growth. *Proceedings of the National Academy of Sciences USA* 99, 5732–5737.
- Zhang, Y.-M., Rock, C.O., 2008. Membrane lipid homeostasis in bacteria. *Nature Reviews Microbiology* 6, 222–233.