INVESTIGATING PHOTOSYNTHETIC STABILITY:

Relation Between Thylakoid Lipid Content and the Stability of the Cytochrome b_6f Complex

Student Author

Marina Mehling is a biological engineering student pursuing minors in biochemistry and biotechnology. As an ag and bio engineering ambassador, Mehling organizes industry tours for students and serves the Biological Engineering department as a member of Alpha Epsilon Honors Society. She is a resident assistant in Duhme Hall, a peer mentor for the Honors Engineering Program, an Honors Engineering ambassador, an athlete in Purdue Club Badminton, and the awards chair of the 2020–2021 class of Purdue Mortar Board. Since 2016, Mehling has worked as a lab technician for the U.S. Department of Agriculture and has published research addressing the oil content and viability of biofuel plants. At Purdue, she has spent two years studying the role of lipids on photosynthetic stability for the Cramer Lab. Mehling intends to earn a PhD in bioengineering to continue biodiesel development and implementation as a university professor.

Mentor

W. A. Cramer is a Henry Koffler Distinguished Professor of Biological Sciences at Purdue University. After earning a bachelor's of science at Massachusetts Institute of Technology, he earned a PhD at the University of Chicago and completed a National Science Foundation postdoctoral fellowship at the University of California–San Diego. Cramer is the recipient of the 2017 NIH Research Career Development Award, among other merits: Senior Fellow, European Molecular Biology Organization, University of Amsterdam; McCoy Award, Purdue University; Fellow, Alexander von Humboldt Foundation; Fellow, Guggenheim Foundation, Biophysical Society, American Association for the Advancement of Science; and Charles F. Kettering Award, American Society of Plant Physiology.

http://dx.doi.org/10.7771/2158-4052.1463

Abstract

The cytochrome b_6f complex is an enzyme found in plants, cyanobacteria, and green algae that catalyzes the transport of electrons in the rate-limiting step of oxygenic photosynthesis. This dimeric complex has an extensive lipid architecture that is primarily composed of five distinct lipid classes: monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG), phosphatidyl glycerol (PG), monoglucosyl diacylglycerol (GlcDG), and sulfoquinovosyl diacylglycerol (SQDG). While these lipid classes have been identified, their precise role in the function of the cytochrome complex are only beginning to be understood. Mechanisms describing the relation between thylakoid lipid content on the stability of the b_6f complex are not known.

This study validates the importance of the lipids on cytochrome b_6f dimer formation and stability by showing that SQDG and the synthetic lipids 1,2-dioleoylphosphatidylglycerol and 2-dioleoyl-sn-glycero-3-phosphocholine reduce the temperature dependent rate of monomerization (denaturation) of the native dimer. A novel method of growing the cyanobacterium *Synechococcus* PCC 7002 anaerobically to test the relation between thylakoid lipid content and growth temperature was developed. This method of growing *Synechococcus* greatly reduces the relative SQDG content and increases the relative PG content in thylakoid membranes. The analysis of MGDG, DGDG, PG, GlcDG, and SQDG content in *Synechococcus* cultures grown at 30°C and 33°C revealed that the MGDG content depends inversely on the growth temperature.

Mehling, M. (2020). Investigating photosynthetic stability: Relation between thylakoid lipid content and the stability of the cytochrome b_6f complex. *Journal of Purdue Undergraduate Research, 10*, 43–51.

Keywords

b_6f, thylakoid, lipids, transport of electrons, photosynthesis, *Spinacia oleracea, Synechococcus*

INTRODUCTION

Role of Lipids on the Thermal Stability of the Cytochrome b_6f Complex

The cytochrome b_6f complex, an enzyme found in plants, cyanobacteria, and green algae, catalyzes the transport of electrons in the rate-limiting step of oxygenic photosynthesis. A study of b_6f lipid architecture (Hasan & Cramer, 2014) identified 23 lipid-binding sites per monomer that were proposed to contribute to the stability and activity of the dimeric complex. Sites were located near detergent n-undecyl β-D-maltopyranoside (UDM) molecules. UDM is utilized for purification of the complex and provides cross-linking interactions between two monomer b_6f proteins to form a dimer (Qin, Sharpe, Garavito, & Ferguson-Miller, 2007). The lipids at these sites are thought to augment stability of the dimer (Qin, Hiser, Mulichak, Garavito, & Ferguson-Miller, 2006; Qin et al., 2007). Monomeric b_6f can result from chemical or thermal denaturation, which disrupts the cross-linking interactions facilitated by UDM or lipid molecules. Removing UDM molecules from the complex increases the responsibility that lipids have in maintaining the functionally active, dimeric structure. The b_6f complex without UDM is a prime medium to test the efficacy of different lipids, such as sulfoquinovosyl diacylglycerol (SQDG), on dimer stability. SQDG has been found to augment photosystem II function in the cyanobacterium *Synechocystis* (Minoda et al., 2002). Consequently, SQDG is hypothesized to increase dimer stability and reduce the rate of monomerization that the b_6f complex observes during heat exposure compared to b_6f without UDM alone.

This study aims to provide additional information on the role of lipids in conferring dimer stability and elucidate which specific lipid classes facilitate formation of the b_6f complex. Identifying these lipid classes may reveal a means to catalyze photosynthesis in plants. Increased efficiency in crop growth and resource production poses multiple economic, environmental, and social benefits.

Temperature Dependence of Lipid Content in Photosynthetic Membranes

Previous studies (Ongun & Mudd, 1968; Siebertz & Heinz, 1977) have shown that the lipid content in the photosynthetic blue-green algae *Anabaena variabilis* has a dependence on growth temperature. The content of 16:0 and 16:1 lipid decreases relative to that of 18:1, while the content of 16:1 and 16:2 lipids decreases relative to 18:3 at lower temperatures;

the ratios 16:0, 16:1, and 16:2 are the fatty acid components of the lipids (Siebertz & Heinz, 1977). To identify the cause of this composition change, Naoki and Norio (1982) proposed mechanisms for lipid synthesis and degradation in *Anabaena variabilis* at 22°C and 38°C. Naoki and Norio concluded that at both temperatures, monoglucosyl diacylglycerol (MGDG), phosphatidylglycerol (PG), monoglucosyl diacylglycerol (GlcDG), and SQDG are the main products of lipid biosynthesis. In MGDG, the stearic acid group is desaturated to oleic acid (18:1) and further to linoleic acid (18:2). At 38°C, the stearoyl-palmitoyl species is converted to oleoyl-palmitoyl and then transformed to either linoleoyl-palmitoyl or oleoyl-palmitoleoyl, which are both subsequently converted to leoyl-palmitoleoyl. In contrast, at 22°C, stearoyl-palmitoyl is converted to oleoyl-palmitoyl, linolenyl-palmitoyl, linolenoyl-palmitoyl, and linolenoyl-palmitoleoyl, in this order (Noaki & Norio, 1982). At both temperatures, digalactosyl diacylglycerol (DGDG) is synthesized from these MGDG derivatives and, unlike PG and SQDG, does not experience desaturation. At both temperatures, the stearic acids in these lipids are desaturated to 18:1 and 18:3, but only at 22°C have they been found to be desaturated to 18:2 (Naoki & Norio, 1982). This study aims to determine the pattern of these lipid-based phenomena in *Synechococcus* by analyzing the total membrane lipid content of cultures as a function of growth temperature. In accordance with the findings in *Anabaena variabilis* (Naoki & Norio, 1982), *Synechococcus* cultures grown at higher temperatures are hypothesized to have lower 18:2 content, suggesting that smaller amounts of MGDG lipids are present.

The strain *Synechococcus* PCC 7002 is sensitive to pH change, which can be influenced by either the medium composition or the surrounding air flow (Silva, Sforza, & Bertucco, 2016). To study temperature dependence, an enclosed growth chamber without airflow must be used. Consequently, a novel method of growing *Synnechochus* PCC 7002 was devised to develop cultures of high concentration under appropriate growth conditions.

RESULTS

Addition of 1,2-dioleoylphosphatidylglycerol (DOPG), 2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), and SQDG Lipids Stabilize Dimeric b_6f Complex

Dimeric b_6f complexes without UDM were introduced with either SQDG, the synthetic lipid DOPG, or DOPC. Heating the samples denatured the quaternary structure of some complexes, resulting in b_6f monomers that were distinguished from the dimers in native gel electrophoresis (Figure 1A). Comparing the relative band intensities of each sample lane allowed a monomer-to-dimer ratio to be calculated and plotted (Figure 1B). All the introduced lipids showed competence in stabilization of the complex in its dimeric form.

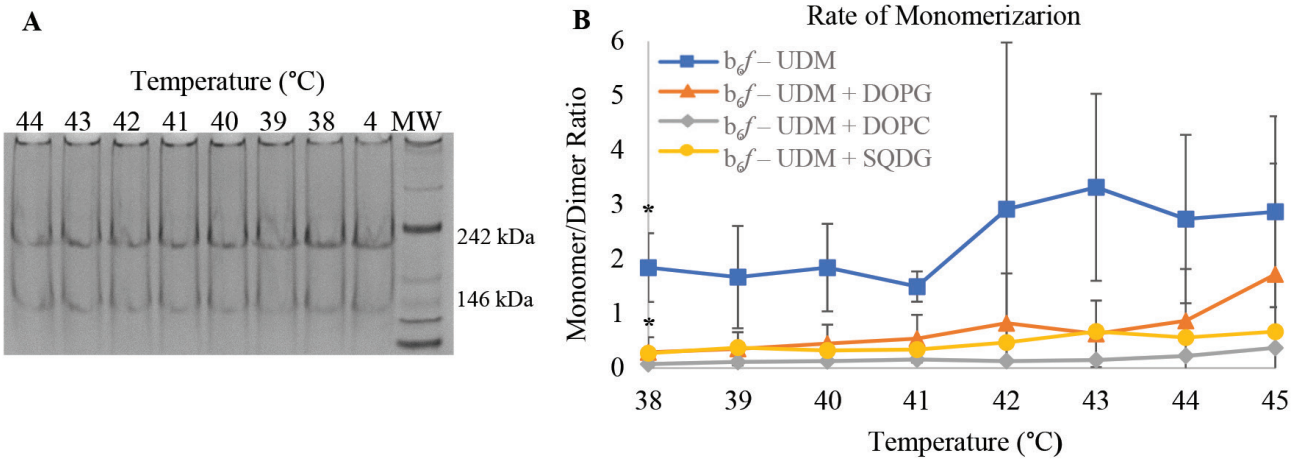

Figure 1. Effects of lipid classes on b_6f monomerization rate.

Note: (A) Example of a native gel image of b_6f complex without UDM or lipid showing dimer (~242 kDa) and monomer (~146 kDa) fractions. MW denotes molecular ruler used to assess protein size. (B) Average monomer-to-dimer ratios (measured through native gel band intensity) plotted with trend lines and appropriate standard deviations for b_6f without lipid (n = 3), with DOPG (n = 2), with DOPC (n = 1), and with SQDG (n = 1). Average ratio at 38°C for DOPG (triangle) is statistically significant (α = .05) compared to the sample average without lipid (blue) as denoted by *.

The average rates of monomerization are visibly greater in samples without lipid compared to samples with DOPG, DOPC, or SQDG (see Figure 1B). The average ratio at 38°C for DOPG is lower from the sample average without lipid, suggesting that the presence of DOPG makes the dimer configuration more favorable for the b_6f complex. Samples containing SQDG displayed the lowest monomer-to-dimer ratios.

Synechococcus Cultures Can Be Grown Anaerobically with 5% Bicarbonate Medium and Regular pH Modification

The ability to alter the growth temperature of *Synechococcus* cultures presented the need to design a growth system in which a temperature-controlled chamber could be used. This prevented the cultures from being grown under air stream as recommended (Yu et al., 2015). To account for the lack of CO_2 supply, $NaHCO_2$ was added to the A-D7 medium to make a 5% solution. Silva et al. (2016) observed that adding bicarbonate to culture media improved the growth efficiency of *Synechococcus.* The addition of $NaHCO_2$ influenced the pH of the growth medium to increase over time due to an imbalance of CO_2 ions, resulting in an increase in the concentration of HCO_3 (Silva et al., 2016):

$$CO_{2(aq)} + H_20 \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$$

The increase in the medium pH caused from bicarbonate addition was experimentally observed to cause premature cell death. Increasing the Tris-HCl concentration from the recommended 0.08 moles (M) (Yu et al., 2015) in the media was hypothesized to stabilize the culture pH. Although increased buffer concentration did reduce alkylation of the medium, premature cell death was observed in cultures containing 0.05 M and 0.1 M concentrations of Tris-HCl (Figure 2A).

The volumetric ratio of 12 M HCl required to stabilize media pH decreased over time for all buffer concentrations (see Figure 2B).

Modifying the pH of 75-ml cultures grown in A-D7 medium with 5% bicarbonate and 0.008-M Tris-HCl on an approximate two-day basis allowed the cultures to reach an optical density at 750 nm (OD_{750}) of 2.3 after 21 days (with an initial OD_{750} between 0.3 and 0.4) (Figure 3).

Growing *Synechococcus* Cultures Without Air Stream Reduces Relative SQDG and Increases Relative PG Content

Performing thin-layer chromatography (TLC) with lipid extractions successfully isolated GlcDG, MGDG, DGDG, SQDG, and PG classes for each sample (Figure 4A). DGDG, SQDG, and PG were distinctly resolved, while some samples had GlcDG and MGDG bands that were in contact (see Figure 4).

Lipid extracts from control cultures (grown in A-D7 medium with air stream at 30°C) were also run on TLC (see Figure 4A and 4B). These samples all presented a spot that corresponds to SQDG lipids (n = 4). None of the cultures grown without air stream in 5% bicarbonate medium presented a visible SQDG spot (n = 2) (see Figure 4B and 4C). Calculating the relative intensities of TLC bands revealed that the control cultures have increased SQDG content (~hundredfold) and decreased PG content (see Figure 4D). While a difference in relative PG amounts is not visible through TLC spotting, software analysis of band intensities indicated a 3% difference in relative abundance (see Figure 4D).

MGDG Content Is Inversely Related to Growth Temperature

The lipid extracts from cultures grown at 33°C presented visibly smaller bands of MGDG content compared to cultures grown at 30°C (see Figure 4C). Calculating the relative intensities of each lipid class validates this observation, since the decrease of MGDG content in the 30°C culture was found to be statistically significant ($\alpha = 0.05$) (see Figure 4E).

DISCUSSION

The lipids DOPG, DOPC, and SQDG help stabilize b_6f dimer formation to mitigate the rate of monomerization when the complex is exposed to heat treatment (see Figure 1B). While all three lipid classes produced average monomer-to-dimer ratios over twofold less than the samples without lipid, only the significance of DOPG can be discussed. Further experimentation was conducted by Bhaduri, Zhang, Erramilli, and and Cramer (2019). The study found that b_6f plus SQDG observed the greatest increase (threefold) of dimer stabilization, while to a lesser degree DOPC and DOPG also reduced monomerization rate. These findings are consistent with the trends presented in Figure 1B.

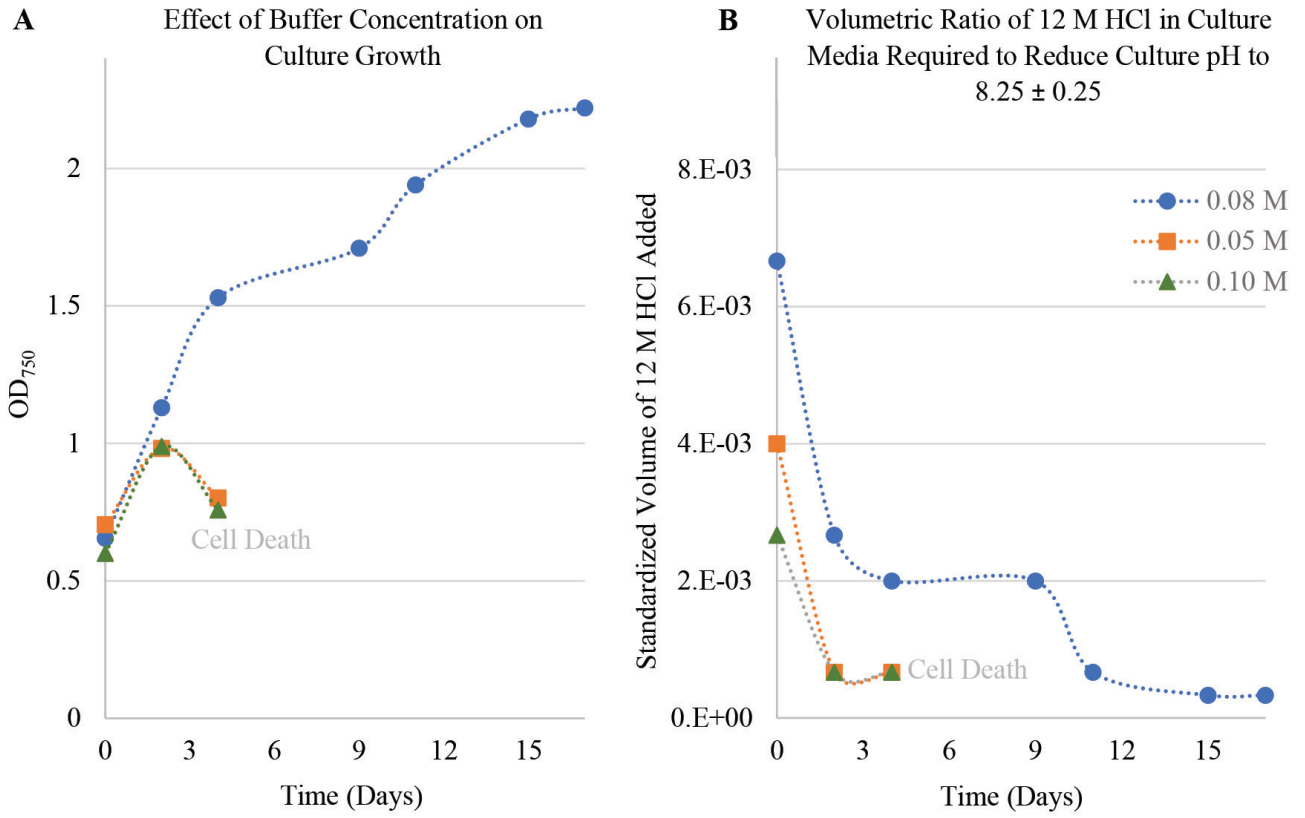

Figure 2. *Synechococcus* development in modified growth media.

Note: Results from inoculating *Synechococcus* cultures at 30°C in 5% bicarbonate A-D7 media with modified Tris-HCl concentration (0.08 M, 0.05 M, and 0.10 M). The pH of each culture was modified after each optical density reading was recorded. Due to prevalent cell death (decrease in optical density, observation of white cell debris in medium, and yellow coloring of culture), the 0.05 M and 0.10 M cultures were disposed of after four days. (A) OD_{750} of each culture over time, n = 1. (B) The volume of 12M HCl required per volume of culture media (ml/ml) required to reduce the culture pH to 8.25 ± 0.25, n = 1. While higher concentrations of Tris-HCl reduced medium alkylation, the original A-D7 concentration of 0.08 M is the most appropriate concentration for *Synechococcus* growth.

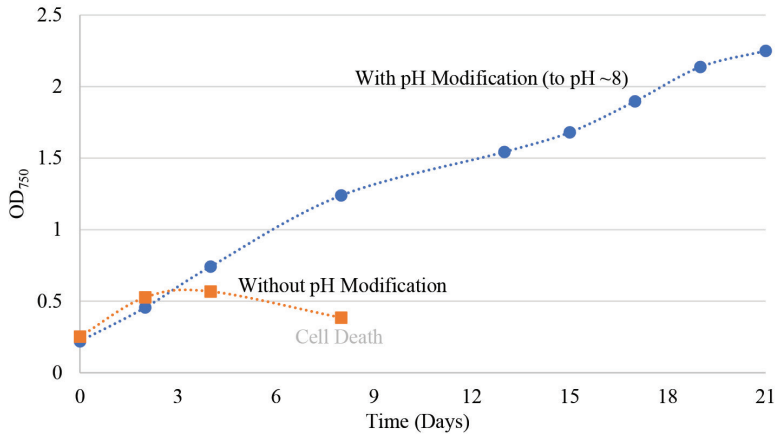

Figure 3. Buffer pH modification required for *Synechococcus* growth.

Note: Results from inoculating *Synechococcus* cultures at 30°C in 5% bicarbonate A-D7 media with and without continual pH modification with 12 M HCl (pH modification occurred at each point on graph along with optical density measurement). Cell death after one week of inoculation was observed in unmodified culture. Cell death was observed through decrease in optical density, observation of white cell debris in medium, and yellow coloring of culture. The culture with pH modification experienced regular pH testing with the addition of 12 M HCl to restore the pH to 8.25 ± 0.25; n = 1 for both samples.

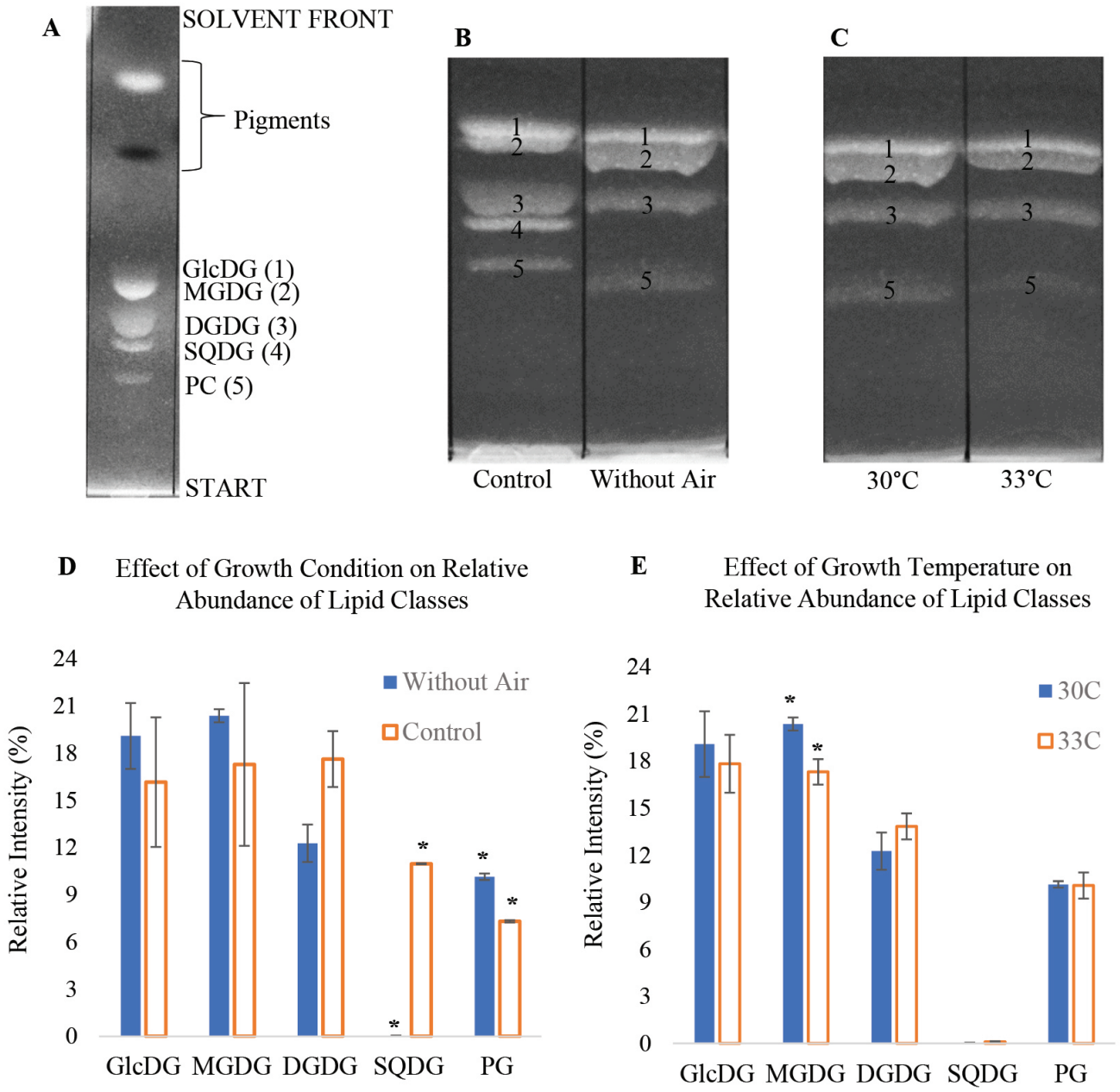

Figure 4. The effect of *Synechococcus* growth condition on lipid composition.

Note: (A) Representative TLC image of control sample grown under normal conditions (A-D7 medium with air stream at 30°C). Full length of solvent front shown with labels corresponding to the primulin-stained lipid classes. The numbers associated with each lipid class are consistent throughout the figure. (B) Representative image that compares 30 µl of control sample (left) against 30 µl of sample grown at 30°C without air stream in 5% bicarbonate A-D7 medium (right). Lanes are cropped so only lipid classes shown. (C) Compares 30 µl of cultures grown at 30°C and 33°C, both grown without air stream in 5% bicarbonate A-D7 medium. Lanes are cropped so only lipid classes are shown. (D, E) Graphs of relative intensities for each lipid class where pigment intensities were included in total abundance but not shown on graphs. Panel D compares culture growth methods, while panel E compares cultures both grown without air stream at varying temperatures. Asterisks (*) denote statistical inequivalence with α = .05 and n = 2.

Bhaduri et al. (2019) attributed SQDG's ability to stabilize the Rieske subunit, a prosthetic group, of the cytochrome subunit to its consequent success in dimer stabilization. Analysis of the structure of SQDG may also provide insight as to why it is best suited for complex stabilization compared to other lipid classes. According to the product data sheet for purchased SQDG (from Avanti Polar Lipids, Inc.), SQDG contains 45% 16:0 and 50% 18:3 content, which is unique compared to the 99% 18:1 content of synthetic DOPC and DOPG. A detailed study of cytochrome c oxidase and lipid interaction in 13 species (Hasan & Cramer, 2014) characterized strong lipid-membrane binding as the ability for alkyl tails to precisely fit through a network of van der Waals contacts. Thus, anionic SQDG lipid has more unsaturation sites than DOPC and DOPG and may be more apt to charge interactions.

When TLC was performed with lipid extractions, the GlcDG and MGDG fraction displayed poor separation (see Figure 4A). This phenomenon may contribute to the large standard deviations presented in the relative intensity calculations (see Figures 4D and 4E). An investigation of cyanobacterial lipids (Sato & Murata, 1988) notes that the solvent system of acetone, benzene, and water (91:30:18, v:v) was able to separate GlcDG and MGDG on 20-cm plates (the length of the solvent front was not reported). The present study utilized the same solvent system and same-size plate with solvent fronts of approximately 17 cm but did not consistently observe clear separation between GlcDG and MGDG.

Nearly 15% of the thylakoid membrane lipid content is composed of SQDG (Bhaduri et al., 2019). Given that PG constitutes approximately 13% of membrane lipid content, it is unexpected to see no SQDG bands on the TLC plates in the presence of PG spots (see Figures 4B and 4C). SQDG is apparently not required for photosystem II function in *Synechococcus,* unlike its vital role in other cyanobacterial systems such as *Synechocystis* (Minoda et al., 2002). In *Spinacia oleracea,* SQDG was shown to improve b_6f dimer stability when subjected to increased temperature (see Figure 1B), while PG was found to be essential for complex stability (Bhaduri et al., 2019). It is unknown if the cultures grown without air stream in 5% bicarbonate medium experience differences in photosynthetic efficiency or membrane stability compared to control *Synechococcus* due to their lack of SQDG content and approximate 3% increase in PG relative abundance. Although the relative intensity calculations indicate that MGDG content is inversely related to temperature (see Figure 4E), it is unknown if this finding is consistent in *Synechococcus* strains grown in different conditions of medium content or air/oxygen flux.

The observed decrease in relative MGDG content for cultures grown at 33°C (see Figures 4C and 4E) supports the initial hypothesis that the temperature dependence of lipid content in *Synechococcus* is consistent with that of the cyanobacterium *Anabaena variabilis,* where cultures grown at higher temperatures desaturate 18:1 to 18:2 for MGDG synthesis at lower rates (Naoki & Norio, 1982). The results from the present study cannot be compared to reported alterations in *Anabaena variabilis* fatty acid composition (Naoki & Norio, 1982) without analysis of the fatty acid classes. An investigation of *Chlamydomonas reinhardtii* and *Chlorella* strains (Kobayashi et al., 2013) used the synthetic lipid 17:0 as an internal standard to quantify MGDG and DGDG through TLC and gas chromatography methods. Quantitation of *Synechococcus* lipid content could potentially also use 17:0 as an internal standard if preliminary experiments show that it migrates distinctly from the five *Synechococcus* lipid classes in TLC.

METHODS

Isolation of Cytochrome b_6f From Spinach and Lipid Introduction

Commercial organic spinach was used for of cytochrome b_6f extraction as previously described (Kobayashi et al., 2013). Dimeric and monomeric complexes were fractionated in a sucrose density gradient and by size-exclusion chromatography (Bhaduri et al., 2019).

The lipids DOPC, DOPG, and SQDG were purchased from Avanti Polar Lipids, Inc., and have the following specifications: DOPC, 18:1 (99%); DOPG, 18:1 (99%); and SQDG, 16:0 (45%) and 18:3 (50%). The lipids were suspended in 10% TNES buffer (10 mM Trizma Base, 100 mM NaCl, 10 mM EDTA, 2% sodium lauryl sulfate) and solubilized through repetitive flash-freezing in liquid N_2 and thawing. After being sonicated for 20 minutes, the lipids were combined to form 10-mM solutions with 1-ml of b_6f aliquots.

Native Gel Electrophoresis

Isolated cytochrome b_6f complexes and lipids (DOPC, DOPG, and SQDG) were suspended in TNES-UDM buffer (pH 8.0) to make a 5-µM sample solution. For each thermal denaturation experiment,

a heating block was used to expose 1-ml samples to temperatures in a range of 42–62°C for a duration of 5 minutes.

The samples were then run on 4–12% gradient native gels, as outlined in a previous publication (Bhaduri et al., 2019). Fiji/ImageJ software was used to calculate a monomer: dimer ratios for each sample through band intensity measurement (Schindelin et al., 2012).

Inoculating *Synechococcus* Cultures

Medium A-D7—medium A (Yu et al., 2015) without $NaVO_3$ but with D7 micronutrients (Stevens, Patterson, & Myers, 1973)—for *Synechococcus* PCC 7002 was prepared. To account for a growth chamber in which air needed for growth cannot be supplied, sodium bicarbonate was added to an aliquot of the medium to create a 5% bicarbonate solution. From this aliquot, an additional three aliquots were created. The amount of Tris-HCl in each solution was varied so that three A-D7 + 5% bicarbonate mediums were made with Tris-HCl molarities of 0.008 (standard), 0.05, and 0.1. Sterile flasks were filled with 75 ml of the appropriate A-D7 media and placed in a constant-light (100 microeinsteins) Algatertron Photon Systems Instruments temperature-controlled growth chamber. Cultures were inoculated from medium A plates (Stevens et al., 1973; Yu et al., 2015) so that the initial OD_{750} was between 0.2 and 0.3. The cultures were shaken at 150 rpm. To test the pH of each culture when necessary, a sterile pipet was used to place culture solutions on BAKER-pHIX 4.5-10 pH strips. To lower the pH of the cultures to 8.25 ± 0.25, 12M HCl was used.

Lipid Extractions from *Synechococcus* and Thin-Layer Chromatography

Total lipid extractions from wild-type nolstock *Synechococcus* were performed using a methanol-chloroform method (Bligh & Dyer, 1959). The lipid extraction procedure was performed on 60-ml cultures with an OD_{750} of 2.3. The final yield was suspended in 3 ml of 1:1 (v:v) chloroform and methanol.

TLC was implemented to separate the five major classes of lipids in cyanobacteria (MGDG, GlcDG, DGDG, PG, and SQDG). The samples were spotted onto Merck KGaA 2.5 by 20-cm silica gel 60 plates. A solvent system of acetone, benzene, and water (91:30:18, v:v) was used in accordance with a study (Sato & Murata, 1988) that isolated these lipid classes from the cyanobacterium *Anabaena variabilis*. The bands were stained with a 2% primulin in acetone solution using a glass sprayer and visualized under ultraviolet light at 366 nm.

Images of the TLC plates were analyzed using Fiji/ImageJ software (Schindelin et al., 2012) to determine the total signal intensity of each lane and each of its individual bands. Dividing the total lane intensity by band intensity allowed for the relative intensity of each lipid class to be reported as a percent.

Determining Statistical Significance

All statistical calculations were performed using a significance (α) of 0.05 with a two-tailed t-test that assumed sample homoscedasticity (Montgomery, Runger, & Hubele, 2011).

ACKNOWLEDGMENTS

This study would not be possible without mentoring from W. A. Cramer and grant support from the Deptartment of Energy (DOE DE-SC00118238). The author would like to thank S. Bhaduri for performing complex b_6f isolations and developing the methodology for introducing lipids to the complex and the monomerization experiment. Gratitude is also extended to Professor Sujith Puthiyaveetil, Gilbert Kayanja, Jillian Ness, and Dr. Stanislav Zakharov for their guidance and consultation and to Professor C. Chapple for use of equipment.

REFERENCES

Bhaduri, S., Zhang, H., Erramilli, S., & Cramer, W. A. (2019). Structural and functional contributions of lipids to the stability and activity of the photosynthetic cytochrome b_6f lipoprotein complex. *Journal of Biological Chemistry, 294*(47), 17758–17767. https://doi.org/10.1074/jbc.ra119.009331

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biological Chemistry, 37*(1), 911–917. https://doi.org/10.1139/y59-099

Hasan, S. S., & Cramer, W. (2014). Internal lipid architecture of the hetero-oligomeric cytochrome b_6f complex. *Structure, 22*(7), 1008–1015. https://doi.org/10.2210/pdb4ogq/pdb

Kobayashi, N., Noel, E. A., Barnes, A., Rosenberg, J., Dirusso, C., Black, P., & Oyler, G. A. (2013). Rapid detection and quantification of triacylglycerol by HPLC-ELSD in *Chlamydomonas reinhardtii* and *Chlorella* strains. *Lipids, 48*(10), 1035–1049. https://doi.org/10.1007/s11745-013-3828-9

Minoda, A., Sato, N., Nozaki, H., Okada, K., Takahashi, H., Sonoike, K., & Tsuzuki, M. (2002). Role of sulfoquinovosyl diacylglycerol for the maintenance of photosystem II in *Chlamydomonas reinhardtii. European Journal of Biological Chemistry, 269*, 2353–2358.

Montgomery, D. C., Runger, G. C., & Hubele, N. F. (2011). *Engineering Statistics* (5th ed.). Hoboken, NJ: Wiley.

Naoki, S., & Norio, M. (1982). Lipid biosynthesis in the blue-green alga, *Anabaena variabilis. BBA—Lipids and Lipid Metabolism, 710*(3), 271–278. https://doi.org/10.1016/0005-2760(82)90109-6

Ongun, A., & Mudd, J. B. (1968). Biosynthesis of galactolipids in plants. *Journal of Biological Chemistry, 243*(7), 1558–1566.
Qin, L., Hiser, C., Mulichak, A., Garavito, R. M., & Ferguson-Miller, S. (2006). Identification of conserved lipid/detergent-binding sites in a high-resolution structure of the membrane protein cytochrome c oxidase. *Proceedings of the National Academy of Sciences of the United States of America, 103,* 16117–16122.
Qin, L., Sharpe, M. A., Garavito, R. M., & Ferguson-Miller, S. (2007). Conserved lipid-binding sites in membrane proteins: A focus on cytochrome c oxidase. *Current Opinion Structural Biology, 17,* 444–450.
Sato, N., & Murata, N. (1988). Membrane lipids. *Methods in Enzymology Cyanobacteria,* 251–259. https://doi.org/10.1016/0076-6879(88)67027-3
Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., et al. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods, 9*(7), 676–682. PMID 22743772, https://doi.org/10.1038/nmeth.2019
Siebertz, M., & Heinz, E. (1977). Hoppe-Seyier's Z. *Physical Chemistry, 358,* 27–34.
Silva, C. E. D. F., Sforza, E., & Bertucco, A. (2016). Effects of pH and carbon source on *Synechococcus* PCC 7002 cultivation: Biomass and carbohydrate production with different strategies for pH control. *Biochemistry and Biophysics, 181*(2), 682–698. https://doi.org/10.1007/s12010-016-2241-2
Stevens, S. E., Patterson, C. O. P., & Myers, J. (1973). The production of hydrogen peroxide by blue-green algae: A survey. *Journal of Physical Chemistry, 9,* 427–430.
Yu, J., Liberton, M., Cliften, P. F., Head, R. D., Jacobs, J. M., Smith, R. D., et al. (2015). *Synechococcus* elongatus UTEX 2973, a fast-growing cyanobacterial chassis for biosynthesis using light and CO2. *Scientific Reports, 5*(8132). https://doi.org/10.1038/srep08132