Light-Dependent Changes in the Lipid and Fatty Acid Composition of Phycocyanin-Free Photosynthetic Lamellae of *Synechococcus*

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The growth of *Synechococcus* at different intensities of white and red light caused changes in the pigment composition. The ratio of chlorophyll a to phycocyanin varied from 1:8,2 in LWLI-grown cells to 1:1,4 in cells grown at HWLI and to 1:15,7 in cultures exposed to HRLI. Acyl lipids were quantitatively determined and fatty acids of the individual lipid classes analysed by GLC. Phycocyanin-free photosynthetic lamellae were obtained by fractional centrifugation. No variation was found in the acyl lipid composition of the membrane preparations. These all contained MGDG, DGDG, SQDG and PG as components. In all the lipids investigated, palmitic, hexadecenoic and octadecenoic acids made up to more than 90% of total fatty acids. The pattern of these major components within the lipids from the different cultures depended on the light used. No large differences were detected between zones obtained from LWLI and HRLI isolated membranes, whereas density gradient centrifugation of those from HWLI-grown cells resulted in a completely different pattern of bands. The variations in lipid and fatty acid composition are discussed with respect to changes observed in lipid composition of whole cells and the results reported on temperature dependent shifts in lipid fluidity in cyanobacteria.

Introduction

The effect of growth temperature on fatty acid composition in cyanobacteria was first investigated by Holton *et al.* [1]. Using a thermophilic *Synechococcus lividus* strain isolated from thermal springs, Fork *et al.* [2] confirmed these authors' findings that growth at a higher temperature decreases the content of unsaturated fatty acids. By lowering the growth temperature from 55 to 38 °C, a decrease in SQDG and PG bound stearic acid occured, while hexadecenoic and octadecenoic acids increased. In both MGDG and DGDG, palmitic and stearic acid decreased while hexadecenoic acid increased [2]. Sato *et al.* [3] who investigated growth temperature influence on lipid and fatty acid composition in *Anacystis nidulans* and *Anabaena variabilis* in a range from 38 to 22 °C, reported that in *Anacystis* cells temperature dependent changes in acyl lipid composition occured. Their acid composition are different in the two positions of the lipids.

In model membrane studies it has been established that a correlation between phase transition temperature and lipid species as well as the degree of unsaturation at fatty acids exists [4–6]. Murata *et al.* [7] who investigated the relationship between the transition of the physical phase of membrane lipids and photosynthetic parameters found that when *Anacystis nidulans* was grown at 28 °C instead of 38 °C transition occured at a lower temperature. The dependence of the phase transition temperature on fatty acid desaturation has also been reported for several other biological membranes [8–12].

Studies on *Synechococcus*, grown to obtain different pigment ratios [13], showed that light as an environmental factor influences the acyl lipid composition as well as the fatty acid distribution. Especially using high white light (HWLI) caused in the fatty acid content which were comparable to those observed when cyanobacteria were grown at higher temperatures. The purpose of the following work was to determine whether the increase in the ratio of saturated to unsaturated fatty acids observed by Döhler and Datz [13] in whole cells of *Synechococcus* grown at high white light, is characteristic for lipids of photosynthetic lamellae.

Materials and Methods

Organism and growth conditions. Synechococcus (former *Anacystis nidulans, strain L 1402-1*) obtained from the Culture Collection of the Institute for Plant Physiology, Göttingen, W.-Germany, was

Abbreviations: MGDG, DGDG, SQDG, monogalactosyl-, digalactosyl-, sulphoquinovosyl diacylglycerol; PG, phosphatidylglycerol; LWLI, low white light; HWLI, high white light; HRLI, high red light.

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grown at two different intensities of white light, 0.6 and 30.8×10^3 μW/cm², and at one of red light (20×10^3 μW/cm², above 650 nm) as described by Döhler and Datz [13].

Membrane preparation and lipid extraction. Cyanobacteria were harvested 3 h after beginning of the light period, washed with 0.1 M potassium phosphate buffer, pH 7.2, and resuspended in 0.1 M borate buffer, pH 8.5. Cells were ruptured with a Branson Sonifier (Model S-75), and phycocyanin-free photosynthetic lamellae were obtained from the homogenates by fractional centrifugation as reported by Löffelhardt [14]. Lipids were extracted from the green sediments using the method of Tevini [15].

Fatty acid analysis. Fatty acid composition of membrane lipids was determined by gas-liquid chromatography. Crude lipid extracts were purified by thin-layer chromatography using the solvent system of Pohl *et al.* [16]. Fatty acid methyl esters were formed from the individual acyl lipids after Müller and Göke [17]. The GLC analyses were carried out using a Varian 3700 Model gas chromatography with a 2 m glass column (1/4″) packed with 10% DEGS on chromosorb G (aw, dmcs, 80–100 mesh) in the conditions earlier mentioned [13]. Chromatograms were quantitated using a Perkin Elmer M 3 integrator.

Analytical methods. The quantities of lipids were determined in separated fractions by estimating glycolipid sugar content [18] and PG-bound phosphorus as described by Fiske and Subbarow [19] and Debuch *et al.* [20]. Calibration curves performed with pure lipids (MGDG, DGDG and PG obtained from Serva, Heidelberg) were used to establishe molar values. Dry weight determination and estimation of pigment contents were carried out as previously described [21]. The extinction coefficients reported by Myers and Kratz [22] were applied for pigment calculation.

Density gradient centrifugation. Phycocyanin-free photosynthetic lamellae were gently suspended in a

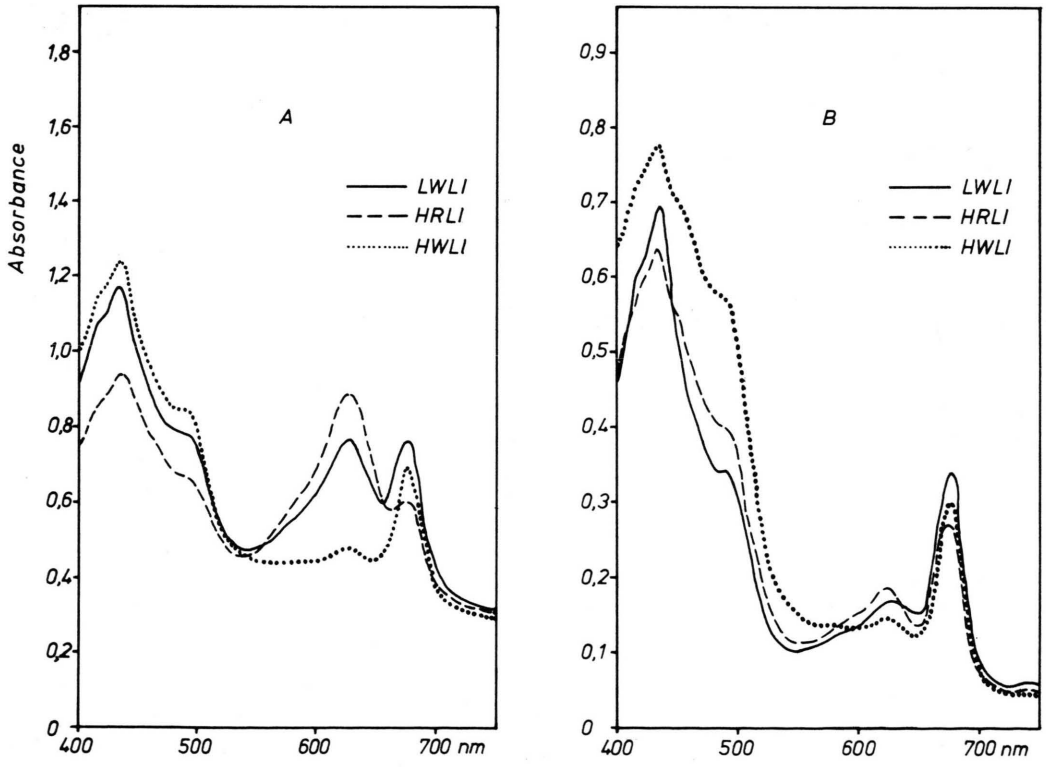

Fig. 1. Absorption spectra of *Synechococcus* grown at different illumination conditions. *In vivo* spectra (A) measured at 20 °C using a Shimadzu UV-180 spectrophotometer. The different cultures were not adjusted to equal cell numbers. Curves are corrected for scattering at 750 nm. (B) Absorption spectra membrane fragments obtained from the three cultures as described in Materials and Methods.

grinding medium as described by Löffelhardt [23] and layered on a discontinous sucrose gradient consisting of 2.5 ml 65% (w/w) sucrose, 2.5 ml 55% sucrose, 2.5 ml 50% sucrose, 2.0 ml 44% sucrose and 3.0 ml 40% sucrose (modified after Löffelhardt [14]). Centrifugations were performed with a Beckmann centrifuge (Model L 5-65, Sw 40 TI-rotor) at 100000 × *g* for 3 h.

Absorption spectra. The spectral characteristics of the differently cultured cyanobacteria and their corresponding membrane fragments were recorded with Shimadzu UV-180 spectrophotometer. The bands obtained by density gradient centrifugation were analysed for their relative protein content with the Isco Type 6 Optical Unit connected to an Isco Gradient Fractionator. No lipid analysis were carried out with the obtained bands.

Results

Effect of light on pigment composition

Synechococcus was grown at two levels of white light and at one of red light of high intensity at a wave-length above 650 nm. Under these conditions the total pigment content, per mg dry cell weight, decreased from 260.9 µg as measured in HRLI-grown cells, to 109.4 µg and 12.4 µg in cells grown at LWLI respectively. The *in vivo* absorption spectra (Fig. 1 A) of the different cultures make it clear, that the selected light influences the ratio of pigment components to each other, especially of chorophyll a to phycocyanin. While absorption maxima of chlorophyll a (678 nm) are equal in LWLI-grown cells, chlorophyll a absorption in HWLI-grown cells is slightly, and that of phycocyanin distinctly diminished. Culturing cyanobacteria in HRLI in comparison led to a pronounced increase in the phycocyanin absorption maximum. The chlorophyll a peak here appears considerably reduced. These findings correspond to results reported by several other authors [24–27]. Calculated from data published in another work [13] the chlorophyll a to phycocyanin ratio of the cultures investigated varied from 1:8.2 (LWLI-grown cells) to 1:1.4 in cells grown at HWLI and to 1:15.7 in cells exposed to HRLI. In the red region of the absorption spectra, photosynthetic lamellae isolated from the three cultures differ only slightly (Fig. 1 B). Green particles from HWLI-grown cells however contained more carotenoids (490 nm) relative to chlorophyll a than did membrane obtained from both the other cultures. Within this HWLI-culture the ratio of chlorophyll a to total carotenoids was measured as 1:1.3, whereas corresponding data in LWLI and HRLI-cultures amounted to 1:0.8 and to 1:0.9 [13]. This confirms results reported by Öquist. He too measured a higher carotenoid to chlorophyll a proportion in the absorption spectra of phycocyanin-

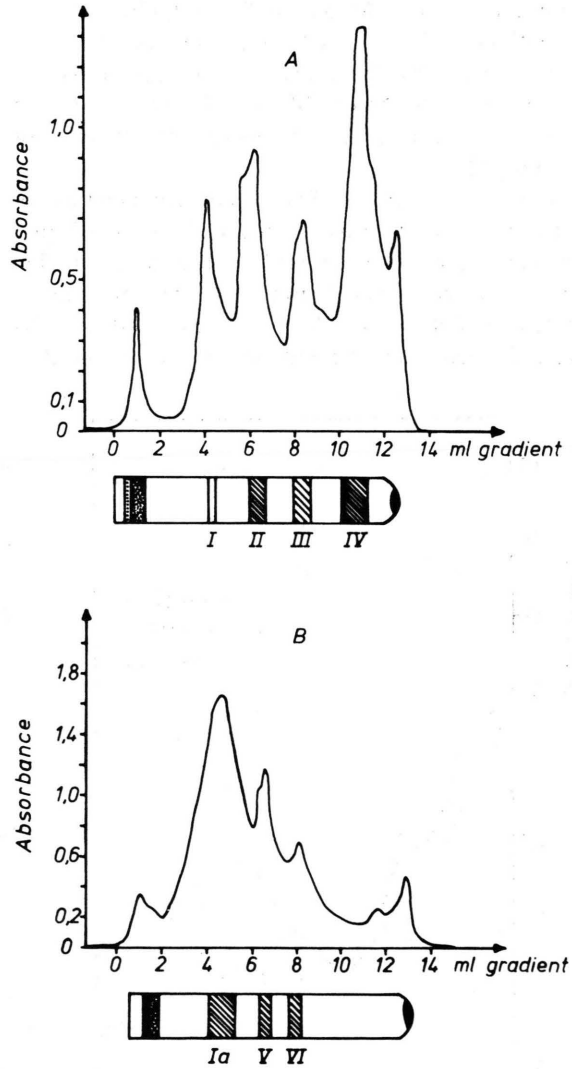

Fig. 2. Band patterns and protein spectra of photosynthetic membranes of *Synechococcus* obtained by means of density gradient centrifugations followed by protein measurement with an ultra-violet-filter equipped flow cell. LWLI and HRLI-isolated membrane fragments yielded in an identical band pattern, (A) representing LWLI-results. The results obtained with HWLI-grown cells isolated lamellae are given in (B). Band colours, I, light green; II, V, VI, green; Ia, III, IV, dark green.

Table I. Acyl lipids composition of photosynthetic lamellae obtained from differently pigmented *Synechococcus* cultures. All µmol values are per 100 mg dry weight and represent the means of 4 to 6 determinations.

Acyl lipids	Illumination conditions					
	HRLI		LWLI		HWLI	
	µmol	% of total	µmol	% of total	µmol	% of total
MGDG	1.16	48.7	0.85	52.1	0.35	47.9
DGDG	0.60	25.2	0.36	22.1	0.20	27.4
SQDG	0.41	17.2	0.27	16.6	0.10	17.7
PG	0.21	8.8	0.15	9.2	0.08	11.0
Total	2.38		1.63		0.73	

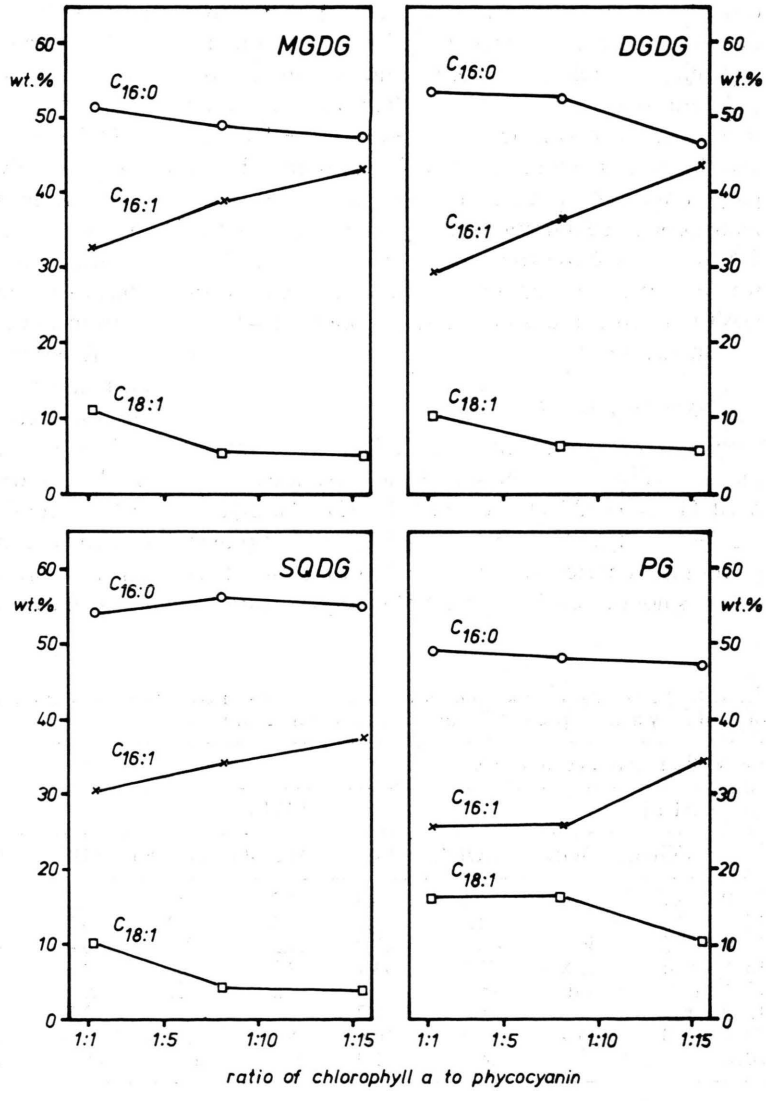

Fig. 3. Changes in membrane lipids fatty acid distribution. Major components plotted against chlorophyll a to phycocyanin ratio of the cultures investigated. Data given in wt.-% of total fatty acids represents the means of 3 to 5 determinations.

free photosynthetic lamellae obtained from HWLI-grown *Anacystis nidulans* cells [28].

Density gradient centrifugation of membranes

Electronic microscope studies (Döhler *et al.*, in press) on differently pigmented *Synechococcus* cells indicate, that cyanobacterial growth under the light conditions used in the present work affects the fine structure of thylakoid membranes. In order to compare the assembly of the photosynthetic lamellae derived from differently grown *Synechococcus* cultures, subunits were prepared by means of centrifugation through discontinuous sucrose gradients. The band patterns obtained from LWLI and HRLI-grown cell membranes were identical (Fig. 2A) both distinctly differing from that of HWLI isolated phycocyanin-free lamellae (Fig. 2B). Through determining the relative protein content using a flow cell (ultra-violet filter, 280 to 310 nm) it was found that a clear cut correlation between protein peaks and the green band patterns exists. In the membrane preparations of HRLI and LWLI-grown cells, the main protein containing constituent was detected in 54% to 58% sucrose (dark-green band IV, Fig. 2A), whereas the one obtained from membranes of HWLI-grown cultures occurred at about the 44% to 46% sucrose level.

Acyl lipid composition

Photosynthetic lamellae investigated contained the acyl lipids characteristic of most cyanobacteria, MGDG (47.9–52.1%), DGDG (22.1–27.4%), SQDG (13.7–17.2%) and PG (8.8–11.0%). The unknown glycolipid reported to occur in HWLI-grown cells [13] was not detected in the membrane preparations. As expected from quantitative pigment data, total acyl lipid concentration decreased from HRLI to HWLI-grown cell membranes. Per 100 mg dry cell weight, 2.38 µmol were measured in HRLI, whereas LWLI and HWLI data accounted for 1.63 and 0.73 µmol respectively (Table I). In comparison with the 2.4 MGDG to DGDG mol ratio found in LWLI-derived membranes, high light irradiated cultures irrespective of light quality possess a decreased content of MGDG relative to DGDG in their membranes (1.9 mol ratio in HRLI and and 1.8 mol ratio in HWLI). Regarding percentage distribution, no difference was measured in the content of negatively charged SQDG and PG lipids using LWLI or HRLI illumination. HWLI conditions on the contrary decreases SQDG level, whereas 11.0% PG content was significantly increased in comparison with HRLI and LWLI irradiance.

Changes in fatty acid composition

GLC analyses of the fatty acids (Table II) prepared from membrane lipids of differently illuminated *Synechococcus* cells showed, that palmitic, hexadecenoic and octadecenoic acids as major components accounted for more than 90% of total fatty acids in all lipids.

Regarding the influence of the particular light on major fatty acid percentage distribution (Fig. 3) equal effects on glycolipid esterified hexadecenoic and octadecenoic acids were noted. In all of these lipids hexadecenoic acid increased from HWLI to HRLI illumination. Glycolopid octadecenoic acid content did not differ in LWLI and HRLI-grown cell membranes (4.4 to 6.6 wt.-%), whereas proportions measured in HWLI were significantly in-

Table II. Fatty acid components of membrane lipids of *Synechococcus*. n.d., not detected; tr., traces. Data given in wt.-% of total fatty acids represent the means of 3 to 5 determinations.

Fatty acid	Illumination conditions											
	HRLI				LWLI				HWLI			
	MGDG	DGDG	SQDG	PG	MGDG	DGDG	SQDG	PG	MGDG	DGDG	SQDG	PG
14:0	0.7	0.6	1.0	0.8	1.2	tr.	tr.	tr.	1.2	2.3	1.4	1.1
14:1	2.3	2.1	1.8	1.8	1.6	1.2	0.8	0.6	1.1	0.9	1.4	1.5
16:0	47.5	46.5	54.9	47.2	49.2	52.7	56.4	48.2	51.5	53.6	54.1	49.2
16:1	43.4	43.8	37.5	34.7	39.3	36.1	34.3	26.6	32.8	29.5	30.3	26.0
17:1	n.d.	n.d.	tr.	0.9	n.d.	n.d.	tr.	0.7	n.d.	n.d.	tr.	1.6
18:0	tr.	tr.	tr.	1.7	1.5	1.9	2.7	5.3	1.3	1.7	1.3	1.9
18:1	5.3	5.9	4.1	10.6	5.5	6.6	4.4	16.5	11.6	10.5	10.3	16.6
20:0	tr.	tr.	tr.	1.4	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.

creased (10.3 to 11.6 wt.-%). Palmitic acid as main constituent in all the lipids investigated was decreased in galactolipids with increasing SQDG was slightly higher in LWLI relative to both high light conditions. Changes in PG fatty acid composition were mainly expressed through the influences on hexadecenoic and octadecenoic acid contents. No differences were measured between HWLI and LWLI irradiation (hexadecenoic acid content amounted to 26.0 and 26.6 wt.-%; octadecenoic acid content amounted to 16.6 and 16.4 wt.-% respectively). HRLI induced increase in hexadecenoic acid level (up to 43.7 wt.-%) was accompanied by a decrease in octadecenoic acid content ot 10.6 wt.-%. Palmitic acid content in phosphatidyl glycerol decrease slightly, similar to the changes measured in galactolipids.

Calculated from major fatty acids wt.-% data given in Fig. 3, an increase in saturated to unsaturated fatty acid ratio was determined for galactolipids as well as phosphatidyl glycerol from HRLI to HWLI illumination. In SQDG this ratio was highest at LWLI irradiation (1.46), whereas high light, irrespective of chosen quality led to a dcrease measured as 1.32 (HRLI) and 1.33 (HWLI) respectively (Table III).

Table III. Ratios of major saturated to unsaturated fatty acids as a function of illumination conditions.

saturated / unsaturated	Acyl lipids											
	MGDG			DGDG			SQDG			PG		
	HRLI	LWLI	HWLI	HRLI	LWLI	HWLI	HRLI	LWLI	HWLI	HRLI	LWLI	HWLI
16:0	0.98	1.10	1.16	0.94	1.23	1.34	1.32	1.46	1.33	1.04	1.12	1.15
16:1 & 18:1												

Discussion

In a recent investigation on lipid and fatty acid distribution in *Synechococcus* cells [13], conditions were reported to achieve phycocyanin deficient and enriched cultures at equal levels of carbon dioxide supply and temperature (35 °C). Results on acyl lipid changes in phycocyanin-free photosynthetic membranes of these cultures presented in this work indicate, that only total lipid variations are comparable to those measured in whole cells. Lipid content decreased parallel to the decreasing pigment content, especially that of phycocyanin. Regarding the proportions of individual membrane acyl lipids, deviations from the pigment composition of low white light grwon cultures were accompanied by a decreased MGDG to DGDG ratio. This result clearly contrasts observations in whole cells, where the proportion of MGDG to DGDG increased with increasing chlorophyll a to phycocyanin ratio [13]. The unknown glycolipid reported to occur in HWLI-grown cells [13] was not found to be located in the phycocyanin-free lamellae obtained from this

culture. Negatively charged membrane lipids percentage distribution was only affected at HWLI-conditions, the SQDG content showing slight reduction relative to that in whole cells, whereas the PG level was slightly increased.

In contrast to differences in acyl lipid contents observed in whole cell and membranes, changes in membrane lipids fatty acid composition were comparable to those measured in whole cells. In general a decrease in palmitic and octadecenoic acid content and in increase in hexadecenoic acid portion parallel to the increasing chlorophyll a to phycocyanin ratio were noted. Only SQDG palmitic acid portions showed no significant changes in photosynthetic lamellae investigated. Expressed as saturated relative to unsaturated fatty acid ratio, an increase in saturation was observed with a decreasing chlorophyll a to phycocyanin ratio in galactolipids and phosphatidyl glycerol. This confirms results obtained with whole cells, in which the SQDG saturated to unsaturated fatty acid ratio also increased, while the pigment ratio decreased [13].

In cyanobacteria temperature dependent changes in fatty acid composition were recently reported [2, 3]. Fork *et al.* [2] using a thermophilic *Synechococcus lividus* strain as well as Sato *et al.* [3] investigating *Anacystis nidulans* reported that lowering growth temperature in general increased the unsaturated fatty acid portion in all lipid classes. Fork *et al.* [2] changing temperature from 55 to 38 °C mentioned that cells grown at 38 °C were yellow-green compared to the usual blue-green colour of thermophilic *Synechococcus* grown at 55 °C. This observation together with the observed fatty acid changes contradicts our results, that deviations from the normal blue-green towards phycocyanin deficient cultures are accompanied by a decrease in unsaturated fatty acids.

Data reported on shifts in *Anacystis nidulans'* fatty acid composition when temperature was lowered from 38 to 22 °C [3], in particular agree with our observations on lipid changes parallel to the increasing pigment ratio from HWLI to HRLI conditions, though *Synechococcus* cultures were grown at equal temperatures. Although Sato *et al.* [3] did not mention light conditions applied in their investigation, the unaltered levels of total acyl lipids and minor alterations in chlorophyll a content reported indicate that pigment composition was not affected by the chosen temperature. Therefore both temperature and light conditions as environmental parameters have to be distinguished in their effects on lipid composition in cyanobacteria.

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[1] R. W. Holton, H. H. Blecker, and M. Onore, Phytochemistry **3,** 595–602 (1964).
[2] D. C. Fork, N. Murata, and N. Sato, Plant Physiol. **63,** 524–530 (1979).
[3] N. Sato, N. Murata, Y. Miura, and N. Ueta, Biochim. Biophys. Acta **572,** 19–28 (1979).
[4] B. D. Ladbrooke and D. Chapman, Chem. Phys. Lipids **3,** 304–356 (1969).
[5] H. Träuble, Naturwissenschaften **58,** 277–284 (1971).
[6] D. Chapman, J. Urbina, and K. M. Keough, J. Biol. Chem. **249,** 2512–2521 (1974).
[7] N. Murata, J. H. Troughton, and D. C. Fork, Plant Physiol. **56,** 508–517 (1975).
[8] S. Eletr and A. D. Keith, Proc. Nat. Acad. Sci. USA **69,** 1353–1357 (1972).
[9] D. M. Engelman, J. Biol. **47,** 115–117 (1970).
[10] D. L. Melchior, H. J. Morowitz, J. M. Sturtevant, and T. Y. Tsong, Biochim. Biophys. Acta **219,** 114–122 (1970).
[11] P. Overath, H. U. Schairer, and W. Stoffel, Proc. Nat. Acad. Sci. USA **67,** 606–612 (1970).
[12] J. K. Raison, J. M. Lyons, R. J. Mehlhorn, and A. D. Keith, J. Biol. Chem. **246,** 4036–4040 (1971).
[13] G. Döhler and G. Datz, Z. Pflanzenphysiol. **100,** 427–435 (1980).
[14] W. Löffelhardt, Z. Naturforschung **31 c,** 693–699 (1976).
[15] M. Tevini, Z. Pflanzenphysiol. **65,** 266–272 (1971).
[16] P. Pohl, H. Glasl, and H. Wagner, J. Chromat. **49,** 488–492 (1970).
[17] B. Müller and G. Göke, Lebensmittelchem. u. gerichtl. Chem. **27,** 165–175 (1973).
[18] P. G. Roughan and R. D. Batt, Anal. Biochem. **22,** 74–88 (1968).
[19] C. H. Fiske and Y. Subbarow, J. Biol. Chem. **66,** 375–400 (1925).
[20] H. Debuch, W. Mertens, and M. Winterfeld, Hoppe Seyler's Z. Physiol. Chem. **349,** 896–902 (1968).
[21] G. Döhler and K.-R. Przybylla, Planta **90,** 163–173 (1970).
[22] J. Myers and W. A. Kratz, J. Gen. Physiol. **39,** 11–22 (1955).
[23] W. Löffelhardt, Hoppe Seyler's Z. Physiol. Chem. **354,** 1006–1012 (1973).
[24] L. W. Jones and J. Myers, J. Physiol. **1,** 7–14 (1965).
[25] G. Öquist, Physiol. Plant. **30,** 38–44 (1974).
[26] G. Döhler, Planta **131,** 129–133 (1976).
[27] J. C. Goedheer, Photosynthetica **10,** 411–422 (1976).
[28] G. Öquist, Physiol. Plant. **30,** 45–48 (1974).