Effect of Light Intensity upon Lipid Composition of *Nitzschia closterium* (*Cylindrotheca fusiformis*)[1]

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ABSTRACT

Total fatty acid, total sterol, fatty acids of specific lipid classes, and unsaturated fatty acids produced in *Nitzschia closterium* were compared qualitatively and quantitatively as a function of changes in light intensity. Increased levels of total fatty acids were observed in cells grown at high light intensity when compared to cells grown at low light intensity. However, the percentage of unsaturated fatty acid decreased under high light conditions. Fatty acid analysis of triglyceride and 1,3 diglyceride fractions indicated an increase in levels of fatty acid at high light intensity when compared to low light intensity, while levels of polar lipid fatty acids increased at low light intensity. These analyses can be taken to indicate an increase in triglyceride and diglyceride at high light and a decrease in polar lipid at high light. Levels of free fatty acids did not differ significantly with light intensity. The levels of total sterol also were unaffected by changes in light intensity. However, levels of sterol isolated as free sterol and sterol associated in a yet unknown manner in the polar lipid fraction varied with changes in light intensity. Levels of polar lipid sterol increased at high light intensity compared to low light intensity, while the opposite was true for free sterol. The greatest percentage of total sterol was found in the polar lipid class regardless of light intensity.

INTRODUCTION

Little information is available concerning the effect of light intensity upon total lipid composition and composition of specific lipid fractions in algae. What information is available has been in relation to lipid changes in the green alga *Chlorella* and the phytoflagellate *Euglena* when grown heterotropically, photoheterotrophically, or photoauxotropically (1-3). However, in *Euglena gracilis* (3) decreases in chlorophyll and total lipid were observed with increased light intensity when cells were grown photoauxotropically. Increased percentages of some polyunsaturated fatty acids occurred with increased light intensity.

Changes in total lipid and sterol found in natural populations of red and brown algae have been correlated with seasonal changes in sunlight and depth distribution at which the organisms were collected (4,5). Generally, more lipids and sterols were present when algae were collected in the summer months and in shallow water. Increases in sterol concentration also have been observed for laboratory cultures of *Chlorella* when grown at increased light intensity (6). Brandt, et al., (7) found an unidentified bound form of sterol in *E. gracilis* Z and noted that the bound sterol decreased and free sterol increased in light grown cultures, while the reverse was true of dark grown cultures.

Apparently, no studies have been conducted with diatoms in relation to the effect of light intensity upon lipids and specific lipid fraction composition. In view of the role of lipid components in functioning of the photosynthetic apparatus in plants and utilization of data on sterol (8) and fatty acid (9) composition in taxonomic and phytogenetic studies, it is essential to understand how environmental factors affect lipid composition both qualitatively and quantitatively.

EXPERIMENTAL PROCEDURES

Axenic cultures of the diatom *Nitzschia closterium* (*Cylindrotheca fusiformis*; Indiana Culture Collection no. 640) were grown in synthetic seawater medium as described by Lewin (10). Cells were cultured in 500 ml Pyrex carrot tubes at 17 C for 7 days. Both low and high light (LL and HL) intensity cultures were grown simultaneously utilizing fluorescent illumination of 200 and 2000 foot-candles. The lower intensity was achieved by blocking part of the illumination with fine meshed wire screen. Cultures were inoculated with cells adapted for 4 days to the 2000 foot-candle intensity and bubbled with 1% CO_2 in air. Cells were harvested by continuous centrifugation, freeze-dried, and dry wts determined.

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Total lipids were obtained by Soxhlet extraction using chloroform-methanol (2:1, v/v) for 24 hr. The extract was dried, redissolved in chloroform, and filtered through Whatman no. 1 filter paper. The filtrate was evaporated to dryness and the dried residue weighed as the total lipid. Lipid classes were separated utilizing a thin layer chromatographic (TLC) technique outlined by Ginger and Fairbairn (11). Lipid classes corresponding to authentic free sterol, free fatty acid (FFA), 1,3 diglyceride (DG), triglyceride, (TRIG), and polar lipid (PL) (origin) were eluted from TLC plates with diethyl ether. DG plus free sterol (these do not separate), TRIG, and PL bands were refluxed with 20% KOH in methanol (1 mg KOH/mg lipid) for 45 min. The samples were acidified with 6N HCl and partioned with n-hexane, evaporated, and subjected to TLC, as previously described. Fatty acid methyl esters and sterols were eluted from TLC plates and analyzed qualitatively and quantitatively by gas liquid chromatography (GLC) utilizing a 15% Hi-EFF 1BP and a 3% SE-30 column, respectively.

Total fatty acid and sterol analysis was performed as in the above procedure beginning with saponification of an aliquot of the total lipid sample.

RESULTS AND DISCUSSION

Relative quantities of various lipid fractions were compared in *N. closterium* when grown at HL and LL intensities (Table I).

Under HL intensity, the percentage of fatty acid was nearly twice that at LL, although the total lipid at HL intensity increased only 2% above the lipid concentration at LL intensity. The concentration of total sterol remained the same at HL and LL intensities. Lipid fraction analysis of PL, DG, TRIG, and FFA (Table I) indicated the most obvious changes occurred in TRIG fatty acids. At HL intensity, more than 5 times as much fatty acid was observed than at LL intensity. However, in the PL fatty acid more than twice as much fatty acid was observed at LL intensity than at HL intensity. Low concentrations of fatty acids were found in the form of DG and FFA. No significant differences were observed in FFA concentration as a function of light intensity. Differences observed for DG were small considering the total fatty acid concentrations.

Table II illustrates the concentration of

TABLE I

Effects of Light Intensity upon Lipid Composition in *Nitzschia closterium*[a]

Lipid fraction	HL[b]	LL[c]
Polar lipid fatty acid	2.6	5.4
Free fatty acid	0.3	0.4
Diglyceride fatty acid	0.5	0.1
Triglyceride fatty acid	9.2	1.7
Polar lipid sterol	0.4	0.3
Free sterol	0.1	0.2
Total unsaturated fatty acid	8.3	5.8
Total saturated fatty acid	4.3	1.8
Total fatty acid	12.6	7.6
Total sterol	0.5	0.5
Total lipid	22.8	20.8

[a]Average of two experiments; data expressed as percent of dry wt.

[b]HL = high light.

[c]LL = low light.

TABLE II

Effect of Light Intensity upon Lipid Fraction Fatty Acid Concentrations in *Nitzschia closterium*[a]

	Lipid fraction[b]									
	PL		DG		FFA		TRIG		Total concentration	
Fatty acid	HL[c]	LL[d]	HL	LL	HL	LL	HL	LL	HL	LL
14:0	2.6	6.5	0.7	<0.1	0.2	0.2	3.8	0.9	7.3	7.6
15:0	---	---	<0.1	<0.1	<0.1	<0.1	---	0.2	Trace	0.2
16:0	7.1	10.0	1.1	0.3	0.5	0.5	27.3	3.9	36.0	14.7
18:0	---	---	<0.1	<0.1	<0.1	0.1	0.3	---	0.3	0.1
16:1	12.3	31.9	1.0	0.2	0.7	0.8	54.0	6.5	68.0	39.4
16:2	0.7	2.9	---	<0.1	<0.1	0.1	1.2	0.3	1.9	3.3
16:3 + 18:1	1.5	10.4	0.7	0.1	0.2	0.4	2.7	2.6	5.4	13.5
18:2	1.1	2.1	0.6	<0.1	<0.1	<0.1	---	0.4	1.7	2.5
18:3	---	---	0.3	<0.1	---	<0.1	---	---	0.3	Trace
20:5	3.9	7.9	2.0	0.1	1.0	1.5	---	2.4	6.9	11.9

[a]Average of two experiments; data expressed as mg of fatty acid/g dry wt.

[b]PL = polar lipid fatty acid, DG = 1,3 diglyceride fatty acid, FFA = free fatty acid, and TRIG = triglyceride fatty acid.

[c]HL = high light.

[d]LL = low light.

TABLE III

Effect of Light Intensity upon Relative Percentages of Fatty Acids in *Nitzschia closterium*[a]

	Lipid fraction[b]							
	PL		DG		FFA		TRIG	
Fatty acid	HL[c]	LL[d]	HL	LL	HL	LL	HL	LL
14:0	8.7	8.5	11.7	5.6	8.4	6.0	3.8	5.3
15:0	---	---	0.7	2.0	1.1	1.3	---	1.2
16:0	25.3	14.7	20.6	30.1	20.4	13.3	30.9	22.4
18:0	---	---	0.6	5.6	2.2	2.3	0.2	---
16:1	43.3	45.2	21.0	25.3	23.9	26.4	61.1	36.4
16:2	1.4	3.7	---	1.0	1.0	3.0	1.3	1.9
16:3 + 18:1	5.1	15.3	9.6	14.8	4.4	11.3	2.7	14.9
18:2	3.7	2.8	8.1	3.5	3.4	1.3	---	2.4
18:3	---	---	2.0	1.8	---	0.2	---	---
20:5	12.5	9.8	25.6	10.3	35.2	34.9	---	15.5

[a]Average of two experiments; data expressed as percent of total fatty acid in each fraction.

[b]PL = polar lipid, DG = 1,3 diglyceride, FFA = free fatty acid, and TRIG = triglyceride.

[c]HL = high light.

[d]LL = low light.

individual fatty acids in *N. closterium* in relation to light intensity. The highest concentrations of fatty acid were found in the TRIG and PL fractions. Increases in concentration of all fatty acids in the PL class were observed for cells grown at LL intensity as compared to HL intensity. The opposite was true for most fatty acids in the TRIG class, except for 15:0 and 18:2, where slight increases were observed at LL intensity. Considerably more 20:5 was produced at LL intensity in this class. Most fatty acids in the DG class increased in concentration at HL intensity when compared to LL intensity. Little difference in concentration of any fatty acid was observed at either light intensity in the FFA class. The most drastic changes in concentration of individual fatty acids in the TRIG class was with respect to increases of 16:1 and 16:0 at HL intensity. More than 8 and 7 times, respectively, of these fatty acids were produced at HL than at LL intensity. However, the same two fatty acids increased in concentration at LL intensity in the PL class, especially 16:1. It is also interesting to note that most 20:5 fatty acid is associated with the PL class and increases in concentration at LL intensity in the PL and TRIG classes. In view of the obvious increased production of fatty acid under HL conditions and the relatively small change in total lipid as a function of light intensity, it is thought that this disparity might be accounted for by significant increases observed visually in the amount of pigments produced at LL intensity.

Table III compares relative percentages of fatty acid in each lipid class as a percentage of total fatty acid in each respective class. The most significant changes in relative fatty acid composition were with respect to 16:0, 16:1, 16:3 + 18:1, and 20:5. The percentage of 16:0 was greater at HL intensity in all lipid classes except DG. The percentage of 16:1 remained unchanged in all lipid classes except TRIG, where higher percentages were observed under HL intensity. Percentages of 16:3 + 18:1 and 20:5 were highest at HL intensity for all lipid classes except for TRIG where 20:5 was higher at LL intensity.

The sterol composition of *N. closterium* was determined in a previous study (12). The predominant sterol found was the 24S isomer of brassicasterol, (24S)-24-methylcholesta-5,22 E-dien-3β-ol. In the present study, sterol was found in *N. closterium* as free sterol, sterol ester, and sterol associated with the PL class in a yet undetermined form. The highest concentrations of sterol were found in the PL class under both HL and LL intensities with the next highest concentration occurring as free sterol. Changes in the concentration of sterol in the form of PL sterol and free sterol appear to be a function of light intensity. At HL intensity, more sterol was observed in the polar lipid class than at LL intensity. The opposite was true for free sterol under the same conditions. A duplicate experiment verified these differences.

In *E. gracilis*, the concentration of total lipid and chlorphyll has been shown to decrease with increased light intensity over a range of 120-610 foot-candles (3). Sharp increases in the percentages of linolenic and 4, 7, 10, 13-hexadecatetraenoic acids were observed with increased light intensity. Correlations between increases in polyunsaturated fatty acids and Hill reaction activity with increased light intensity were suggested. Kates and Volcani (13) in their

analysis of diatom fatty acids have indicated that diatoms are apparently Hill reaction organisms and yet contain only small amounts of linolenic acid. This suggests to them that the degree of unsaturation of a fatty acid may be of importance in the Hill reaction and not a specific fatty acid.

Changes in specific fatty acids reflect, in part, changes in specific lipid fractions. It is of interest from the standpoint of the present study that the only fatty acids which increased significantly in total concentration at HL intensity were 16:0 and 16:1. All other fatty acids decreased in concentration at HL intensity. Also the percentage of unsaturated fatty acid increased at LL intensity, while total lipid increased at HL intensity. Although direct measurements were not made, cellular pigments comprised a significant portion of the total lipid and appeared to decrease with increased light intensity.

It has long been known that "oil" serves as an energy storage compound in the diatoms (14). The differences observed in the TRIG composition of *N. closterium* apparently reflect the accumulation of these energy storage compounds in response to more optimal environmental conditions.

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