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Effect of Light Intensity on the Lipid Composition of *Euglena gracilis**

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SUMMARY

Cultures of the phytoflagellate *Euglena gracilis* were grown at light intensities varying from 120 to 610 footcandles. With increasing light intensity, the content of chlorophyll and of total lipids declined, whereas the percentage of 4,7,-10,13-hexadecatetraenoic acid and of α-linolenic acid rose sharply. The increased content of the two polyunsaturated fatty acids was even more pronounced in the chloroplast lipids and was greatest when the monogalactosyl glyceride fraction was analyzed separately. In *Euglena*, in spinach, and in *Chlorella vulgaris* all of the C_{16} polyunsaturated acids and the major portion of α-linolenic acid are localized in the monogalactosyl glyceride fraction. The finding that Hill reaction activity and lipid unsaturation show the same response to changes in light intensity is discussed with respect to the possible role of polyunsaturated fatty acids in photosynthetic oxygen evolution.

Current research suggests that in plants and in oxygen-evolving microorganisms, two photochemical systems act cooperatively in the over-all process of photosynthesis (1). A corollary of this concept is that photosynthetic bacteria, which do not evolve oxygen, contain only one of the two photochemical systems.

Characteristically, the photochemical apparatus of all oxygen-evolving organisms is rich in lipids containing mono- and digalactosyl diglycerides, as well as large amounts of polyunsaturated fatty acids (2, 3). On the other hand, in photosynthetic bacteria these chloroplast lipids are relatively rare and polyunsaturated fatty acids entirely absent.[1] Several laboratories have demonstrated the preferential localization of α-linolenic acid in plant or algal galactosyl glycerides, *e.g.* in *Euglena gracilis* (3), in *Anabena variabilis* (5), in alfalfa (6), in runner beans (7), and in spinach (8).

In their work with the phytoflagellate *E. gracilis*, Erwin and Bloch (9) found that only cells grown in the light produce large amounts of α-linolenic acid. In dark-grown cells and various colorless mutants of *Euglena*, α-linolenic acid is a minor component, and the content of galactosyl glycerides is drastically reduced. These observations and various comparative considerations have led to the suggestion that certain polyunsaturated fatty acids are of special significance for the operation of the higher plant type of photosynthesis (10).

Further experiments in support of this hypothesis are described in the present report. It has been found that in growing cultures of photoauxotrophic *E. gracilis*, the content of chlorophyll, of total lipids, and of galactosyl diglycerides increases as a function of light intensity, and that at all light intensities the ratio between the content of chlorophyll and of galactosyl diglyceride in the cells is relatively constant. *Euglena* cells grown at very low light intensities contain drastically reduced amounts of α-linolenic acid and of 4,7,10,13-hexadecatetraenoic acid.

METHODS

Euglena gracilis Z was grown on an inorganic medium (11) in 600-ml columns illuminated with banks of daylight fluorescent lamps at room temperature. The cultures were continuously flushed with a mixture of 5% CO_2-air under sterile conditions. For photoheterotrophic growth the mineral medium was supplemented with sucrose (12). Cell growth was followed by recording optical density at 750 mμ. (Absorption by photosynthetic pigments is minimal at this wave length.) The cells were harvested in the logarithmic phase of growth.

Dry weight and total lipids (extracted with chloroform-methanol, 2:1, v/v) were determined gravimetrically. Chlorophyll was determined by the method of Arnon (13).

Mono- and digalactosyl diglycerides were separated by chromatography on silicic acid columns according to the method of Nichols (14) with the following modification. After elution of neutral lipids and pigments with diethyl ether, the monogalactosyl diglycerides were eluted with 7% (v/v) methanol-ether and the digalactosyl diglycerides with 25% (v/v) methanol-

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[1] Exceptions are the green photosynthetic bacterium *Chloropseudomomas ethylicum* and *Rhodospirillum molischianum*, both of which contain some monogalactosyl diglyceride but not polyenoic acids (4).

TABLE I

Response of photoauxotrophic Euglena gracilis Z to increasing light intensities

Light intensity	Maximum rate of growth	Culture	Chlorophyll	Total lipids	Total galactolipid galactose	Monogalactosyl diglyceride	Digalactosyl diglyceride
footcandles	*ΔO.D./24 hrs*	*mg/100 ml, dry wt*	*μg/mg, dry wt*	*μg/mg, dry wt*	*μg/mg chlorophyll*	*μg/mg, dry wt*	*μg/mg, dry wt*
120	0.005	22.0	76.6	367.0	358.0	40.1	38.0
175	0.037	40.0	60.5	275.0	310.0	33.0	25.0
370	0.065	63.0	51.5	240.0	338.0	31.2	27.0
610	0.081	87.0	43.0	218.0	360.0	21.5	27.6

ether. Individual fractions were further purified by thin layer chromatography in chloroform-methanol-acetic acid, 8:3:1. The galactosyl lipids were located and visualized with diphenylamine reagent. Parallel plates were spotted with 2,7-dichlorofluoresceine, the areas corresponding to mono- and digalactosyl diglycerides were scraped off the plates, and the lipids were eluted with methanol. They were analyzed for galactose and fatty acids according to the procedures reported previously (3).

Chloroplasts were prepared by disrupting *Euglena* cells in a French pressure cell at 3000 p.s.i. in a medium consisting of 0.4 M sucrose in 0.01 M potassium phosphate buffer, pH 7.0. Cell debris was removed by centrifugation at 1000 × *g* for 1 min. The chloroplasts were separated from the supernatant by centrifugation at 4000 × *g* for 15 min, washed once with 0.02 M potassium phosphate buffer, pH 7.0, in 0.05% KCl, and finally suspended in a minimum volume of the same washing solution.

Chloroplast fragments in the supernatant were sedimented by centrifugation at 35,000 × *g* for 30 min and washed once with washing solution. All operations were performed at 0°. The Hill reaction with trichlorophenolindophenol as the electron acceptor was measured with chloroplasts by recording optical density changes at 620 mμ and at pH 7.0.

RESULTS

The effect of raising the light intensity on the rate of growth and on the lipid composition of four cultures of photoauxotrophic *Euglena gracilis* Z is shown in Table I. Over an approximately 5-fold range of light intensity, from 120 to 610 footcandles, the growth rate of the cells increased about 16-fold and the dry weight by a factor of 4.

TABLE II

Fatty acid composition of whole cell lipids from four cultures of photoauxotrophic E. gracilis Z grown at increasing light intensities

Fatty acid	Light intensity			
	120 foot-candles	175 foot-candles	370 foot-candles	610 foot-candles
	% total			
Lauric	1.90	1.80	0.57	1.45
Myristic	4.35	3.95	2.90	3.54
Palmitic	17.80	18.00	13.50	16.90
Hexadecenoic	4.32	4.40	3.60	4.00
Hexadecadienoic	8.00	3.40	3.85	4.75
4,7,10,13-Hexadecatetraenoic	10.70	15.00	16.50	16.60
Stearic	0.68	1 .07	0.46	0.97
Oleic + 7,10,13-hexadecatrienoic	10.30	10.10	8.60	8.85
Linoleic	11.50	7.50	7.30	5.70
γ-Linolenic	2.70	1.98	1.35	0.80
α-Linolenic	26.00	30.00	37.60	33.00
Arachidonic	1.60	1.25	2.09	2.50

TABLE III

Fatty acid composition of chloroplast lipids isolated from four cultures of photoauxotrophic E. gracilis Z grown at increasing light intensities

Fatty acid	Light intensity			
	120 foot-candles	175 foot-candles	370 foot-candles	610 foot-candles
	% total			
Myristic	2.10	1.40	2.56	2.75
Palmitic	39.50	36.20	23.00	25.20
Hexadecenoic	4.25	6.10	6.85	5.05
Hexadecadienoic	5.90	3.50	5.70	3.85
4,7,10,13-Hexadecatetraenoic	8.00	11.70	13.60	14.80
Stearic	0.52	0.48	0.85	0.34
Oleic + 7,10,13-hexadecatrienoic	8.70	6.50	8.70	7.15
Linoleic	9.70	6.10	5.80	6.50
γ-Linolenic ?	0.85	1.40	1.70	1.90
α-Linolenic	20.60	24.80	29.50	31.00

The chlorophyll content was an inverse function of light intensity and, of special interest for the present investigation, the same relationship was observed for the total lipid content of the cells.

The parallel changes in the content of chlorophyll and of total lipids suggested that varying light intensity might primarily affect the typical chloroplast glycolipids. Analysis of the two galactosyl glyceride fractions showed that both the mono- and the digalactosyl diglycerides responded in the same way to light intensity changes as did chlorophyll. From the data in Table I, it can be calculated that the ratio between chlorophyll and galactolipid galactose content remained relatively constant over the range of light intensities tested.

Effect of Light Intensity on Fatty Acids Composition—E. gracilis contains a diverse spectrum of fatty acids including many of the fatty acids typical of either higher plants or higher animals (3, 15). An analysis of the fatty acid composition of *Euglena*, grown under well defined conditions, has shown that the major polyunsaturated fatty acids of photoauxotrophic cells are α-linolenic acid, 4,7,10,13-hexadecatetraenoic acid, and several C_{22} and C_{24} polyunsaturated acids (3), and that the former two acids account for more than half of the total. These analyses have now been extended to include the fatty acid composition of whole cells, of chloroplasts, and of the isolated galactosyl diglycerides.

TABLE IV

Fatty acid composition of monogalactosyl diglyceride fraction from four cultures of photoauxotrophic E. gracilis Z grown at increasing light intensities

Fatty acid	Light intensity			
	120 foot-candles	175 foot-candles	370 foot-candles	610 foot-candles
	% total			
Palmitic	39.60	33.50	12.50	10.80
Hexadecenoic	2.08	2.55	2.40	1.50
Hexadecadienoic	7.35	3.35	5.50	4.68
4,7,10,13-Hexadecatetraenoic	14.90	23.50	27.80	32.20
Stearic	1.38	2.55	0.90	0.82
Oleic + 7,10,13-hexadecatrienoic	5.00	3.35	5.80	4.35
Linoleic	6.95	3.15	5.50	4.75
α-Linolenic	23.20	28.00	40.00	41.00

In agreement with earlier results (3) the data in Table II show that α-linolenic acid and 4,7,10,13-hexadecatetraenoic acid are major fatty acids in photoauxotrophic *Euglena*. The formation of both acids is favored by high light intensities. This is shown more clearly by the lipid analysis of isolated chloroplasts (Table III). At the highest light intensity the percentage of α-linolenate was 50% greater and the percentage of the 4,7,10,13-C_{16} acid 85% greater than at the lowest level of illumination. These increases occurred mainly at the expense of palmitate. The same marked increase of the two polyunsaturated fatty acids and a decline of palmitate are apparent on analysis of the monogalactosyl diglyceride fraction (Table IV). This lipid is located mainly in the chloroplasts.

Whether light intensity has also an effect on the fatty acid composition of the digalactosyl diglyceride fraction has not been examined. It is noteworthy however that this fraction, under given conditions, differs drastically from the monogalactosyl diglycerides in fatty acid composition. This is true not only for *Euglena*, but also for spinach and various green algae (Table V).

It is of interest that the polyunsaturated C_{16} acids are con-

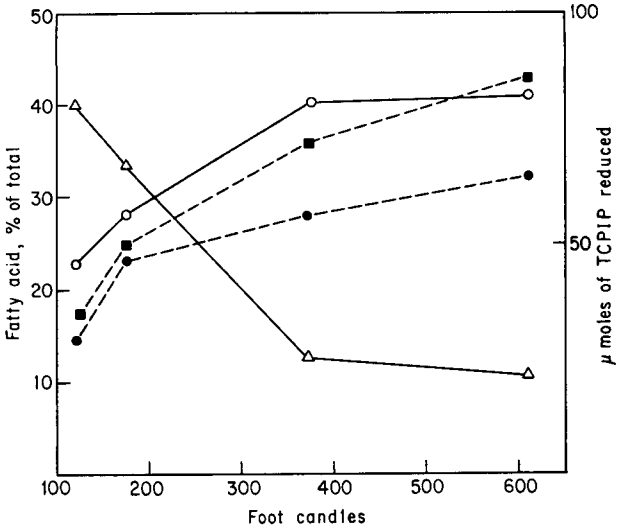

FIG. 1. Effect of light intensity on fatty acid composition of monogalactosyl diglyceride fraction and on Hill reaction activity by whole chloroplasts. *Abscissa*, light intensity; *left ordinate*, fatty acid, in percentage of total in monogalactosyl diglyceride fraction. △——△, palmitic acid; ●- - -●, 4,7,10,13-hexadecatetraenoic acid; ○——○, α-linolenic acid. *Right ordinate*, trichlorphenolindophenol (*TCPIP*) reduced per mg of chlorophyll per hour (■- - -■). The fatty acid values are taken from the data in Table IV.

TABLE V

Fatty acid composition of mono- and digalactosyl diglycerides isolated from photoauxotrophic and photoheterotrophic E. gracilis Z, spinach, and C. vulgaris

Growth conditions for photoauxotrophic and photoheterotrophic *E. gracilis* are described under "Methods." Spinach was purchased from the local market. *Chlorella vulgaris*, wild type, was grown in glass cylinders containing 500 ml of an inorganic medium (16). The cylinders were illuminated in upright position by daylight fluorescent banks which provided an incident light intensity of 500 to 750 footcandles. A mixture of air-5% CO_2 was continuously bubbled through the cylinders under sterile conditions.

Fatty acid	*Euglena*				Spinach		*C. vulgaris*	
	Photoauxotrophic		Photoheterotrophic					
	MGDG[a]	DGDG	MGDG	DGDG	MGDG	DGDG	MGDG	DGDG
	% total		% total		% total		% total	
Myristic	1.7	7.4	—[b]	7.6	—	—	—	—
Pentadecenoic	—	—	—	—	—	10.0	—	—
Palmitic	3.2	15.8	5.0	10.2	1.4	16.4	0.8	30.0
Hexadecenoic	3.3	13.9	6.6	21.3	—	—	1.0	3.0
Hexadecadienoic	6.6	8.3	14.5	20.0	1.6	8.2	36.0	5.7
Stearic	2.4	3.3	0.6	—	—	—	Trace	—
Oleic	6.1	16.5	8.8	26.0	—	10.0	3.5	3.5
7,10,13-Hexadecatrienoic	Trace	—	15.4	—	25.5	—	9.5	—
4,7,10,13-Hexadecatetraenoic	32.3	0.8	8.2	—	—	—	—	—
Linoleic	5.1	11.6	12.2	4.9	1.4	2.8	17.0	37.5
α-Linolenic	39.4	18.0	26.7	9.3	70.0	52.0	31.2	20.0

[a] The abbreviations used are: MGDG, monogalactosyl diglycerides; DGDG, digalactosyl diglycerides.

[b] Dashed lines indicate that the acid was not detectable.

centrated almost exclusively in the monogalactosyl diglyceride fraction. In photoauxotrophic *Euglena*, 4,7,10,13-C_{16} constitutes more than 30% of the monogalactosyl diglyceride fatty acids. Only traces of this acid are present in the form of digalactosyl diglyceride. In photoheterotrophic *Euglena* one finds 7,10,13-C_{16}, as well as 4,7,10,13-C_{16} tetraene, and again the C_{16} polyenoic acids are exclusively localized in the monogalactosyl diglyceride fraction. The preferential localization of the 4,7,10,13-C_{16} tetraene in the monogalactosyl diglyceride fraction of *E. gracilis* has recently been noted also by Rosenberg, Gouaux, and Milch (17).

A high degree of unsaturation is a common feature of the fatty acids of plant galactosyl diglycerides. In some cases (6, 7) these lipids have been found to contain more than 95% α-linolenic acid. It is now becoming clear that the structurally related C_{16} polyenoic acids also contribute in a major way to the unsaturation of algal and plant glycolipids. A specific enrichment of 7,10,13-hexadecatrienoic acid in the monogalactosyl diglyceride of spinach was first reported by Allen *et al.* (8). We have confirmed this result (Table V) and have found the same general pattern in *Chlorella vulgaris* (Table V) and also in *Chlamydomonas reinhardi* and in *Scenedesmus*.[1]

Hill Reaction Activity of Chloroplasts—The increase in Hill reaction activity of isolated chloroplasts per mg of chlorophyll or based on chloroplast, dry weight, was in direct proportion to the increase of light intensity at which the cells were grown. This response shows a high degree of correlation with the level of polyunsaturated fatty acids in the chloroplast monogalactosyl glycerides isolated from the same cells (Fig. 1).

DISCUSSION

The results presented in this investigation show that the well established inverse relationship between light intensity and chlorophyll content in photosynthetic systems holds also for the phytoflagellate *E. gracilis*. Also, with increasing light intensity, both total lipid and galactosyl diglycerides decline in these cells. As a consequence of these parallel changes, the ratio of galactolipids to chlorophyll remains relatively constant at all light intensities. The opposite trend is shown by the degree of unsaturation of *Euglena* fatty acids. The higher the light intensity during the growth of the cells, the greater the concentration of 4,7,10,13-C_{16} and of α-linolenic acid, the major unsaturated fatty acids of the chloroplast galactolipids. Since at high light intensities the content of chlorophyll and of total lipids is low, the net effect of increasing the degree of illumination is to produce cells which are relatively lean but rich in those polyunsaturated fatty acids which are concentrated in the chloroplast galactosyl diglycerides. The fact that in chloroplasts from cells grown at high light intensities Hill reaction activity is also elevated strongly suggests a correlation between competence for oxygen evolution and the degree of unsaturation of chloroplast galactolipids.

A role for polyunsaturated fatty acids, especially α-linolenate in the Hill reaction, was first discussed when it was found that the content of α-linolenate is high in photoauxotrophic *Euglena*, lower in photoheterotrophic cells, and reduced to trace levels in etiolated cells (3). Comparative arguments have served to strengthen this hypothesis (10). More recently, Appleman, Fulco, and Shugarman (16) have found parallel and periodic changes in O_2 evolution and in α-linolenate content on examination of whole cells of *C. vulgaris*. These observations also point to a correlation between Hill reaction activity and content of polyunsaturated fatty acids.

The environmental parameters which we have studied have striking effects not only on α-linolenate content but also on the levels of 4,7,10,13-hexadecatetraenoate. The responses either to light intensity or to changes in growth medium are of similar magnitude for the two polyunsaturated fatty acids. The similarity extends further to the chemical and intracellular localization; both fatty acids are concentrated in the monogalactosyl diglyceride fraction of the chloroplast. Whatever the role of polyunsaturated acids in the Hill reaction, it seems likely that the C_{16} polyenoic acids (7,10,13-C_{16} and 4,7,10,13-C_{16}) are functionally equivalent to α-linolenate. In this context the structural similarity of these fatty acids is worth noting. A terminal carbon chain containing three double bonds at the same distance from the ω carbon of the fatty acid is common to both α-linolenate and the C_{16} polyenoic acids and distinguishes these plant acids from the animal acids, γ-linolenate and arachidonate.

The earlier hypothesis that lipids containing α-linolenate may play a specific role in photosynthetic oxygen evolution (9) is probably not valid, at least in the sense that the function of α-linolenate is essential and specific. Revision of the hypothesis has become necessary because a recent lipid analysis of *Anacystis nidulans* has shown α-linolenate to be absent in this blue-green alga (18). Although *Anacystis* has to date remained the only such exceptional case, it is clear from this example that photosynthetic oxygen evolution can take place in organisms lacking polyunsaturated fatty acids. The blue-green alga *Anabena variabilis*, in contrast to *Anacystis*, contains large amounts of α-linolenate (5), and it therefore becomes of interest to inquire why the lipid chemistry of two representative blue-green algae should differ so markedly. In this respect it may be relevant to cite the comment of Fredricks and Jagendorf (19): "Lamellar fragments of *Anacystis* differ in a number of respects from chloroplasts of higher plants with respect to their Hill reaction activity."

At any rate, granting that there may be some exceptions, it is a striking fact that the photosynthetic organelles of algae and higher plants, but not of photosynthetic bacteria, contain large amounts of α-linolenate and C_{16} polyenoic acids, and that there is a strong correlation between the competence for the Hill reaction and the content of these fatty acids. If perhaps not essential, the plant fatty acids linked to galactosyl glycerides may nevertheless have evolved to provide an especially favorable environment for the electron transport processes in higher plant photosynthesis.

REFERENCES

1. Vernon, L. P., and Avron, M., *Annu. Rev. Biochem.*, **34**, 269 (1965).
2. Benson, A. A., *Annu. Rev. Plant Physiol.*, **15**, 1 (1964).
3. Hulanicka, D., Erwin, J., and Bloch, K., *J. Biol. Chem.*, **239**, 2778 (1964).
4. Constantopoulos, G., and Bloch, K., *J. Bacteriol.*, in press.
5. Levin, E., Lennarz, W. J., and Bloch, K., *Biochim. Biophys. Acta*, **84**, 471 (1964).
6. O'Brien, J. S., and Benson, A. A., *J. Lipid Res.*, **5**, 432 (1964).
7. Sastry, P. S., and Kates, M., *Biochemistry*, **3**, 1271 (1964).
8. Allen, C. F., Good, P., Davis, H. F., and Fowler, S. D., *Biochem. Biophys. Res. Commun.*, **15**, 424 (1964).

9. Erwin, J., and Bloch, K., *Biochem. Z.*, **338,** 496 (1963).
10. Erwin, J., and Bloch, K., *Science*, **143,** 1006 (1964).
11. Cramer, M., and Myers, J., *Arch. Mikrobiol.*, **17,** 384 (1952).
12. Hutner, S. H., Bach, M. K., and Ross, G. I. M., *J. Protozool.*, **3,** 101 (1956).
13. Arnon, D. I., *Plant Physiol.*, **24,** 1 (1949).
14. Nichols, B. W., *Biochim. Biophys. Acta*, **70,** 417 (1963).
15. Korn, E. D., *J. Lipid Res.*, **5,** 352 (1964).
16. Appleman, D., Fulco, A. J., and Shugarman, P. M., *Plant Physiol.*, **41,** 136 (1966).
17. Rosenberg, A., Gouaux, J., and Milch, P., *J. Lipid Res.*, **7,** 733 (1966).
18. Holton, R. W., Blecker, H. H., and Onore, M., *Phytochemistry*, **3,** 595 (1964).
19. Fredricks, W. W., and Jagendorf, A. T., *Arch. Biochem. Biophys.*, **104,** 39 (1964).