THE α-LINOLENIC ACID CONTENT OF SOME PHOTOSYNTHETIC MICROORGANISMS*

J. Erwin† and Konrad Bloch

From the James Bryant Conant Laboratory
Harvard University, Cambridge, Massachusetts

Received August 8, 1962

A survey of the reported natural occurrence of the isomeric α- and γ-octadecatrienoic acids reveals an unusual pattern of distribution. The $\Delta^{9,12,15}$ or α-isomer of linolenic acid is a prominent constituent of plant lipids (Hilditch, 1956; Shorland, 1962) but is not synthetized by higher animals (Mead, 1960) or ciliated protozoa.[1] On the other hand, γ-linolenic acid, the $\Delta^{6,9,12}$ isomer, has been identified as an intermediate in the synthesis of arachidonic acid from linoleic acid in higher animals (Mead, 1960), and it is also the principal fatty acid in certain ciliated protozoa.[1] The γ-acid has been observed in plants only in one case (Riley, 1949).

While investigating the synthesis of unsaturated fatty acids in certain phytoflagellates, we decided to examine the lipid pattern of Euglena gracilis, an organism which has a characteristic dual mode of life. This flagellate can either exist photosynthetically as a "green plant," or it can grow as a colorless "animal-like" organism in the dark (Wolken, 1961). It, therefore, seemed of interest to ascertain whether the change from one mode of existence to the other had any effect on the synthesis of α-linolenic acid and possibly other unsaturated fatty acids in Euglena. For the same purpose, we have analyzed the unsaturated fatty acids of certain chlorophyll-less Euglena "mutants" and of the phytomonad Chlamydomonas reinhardi.

The various strains of Euglena were cultured on a synthetic medium at 25° (Hutner et al, 1956). The medium for the culture of Chlamydomonas has been described (Levine and Ebersold, 1958). Cells were harvested in the logarithmic phase of growth by centrifugation at low speed and were digested by heating in methanolic KOH. The fatty acids were isolated and

* Supported by grants-in-aid from the National Science Foundation, the United States Public Health Service, the Life Insurance Medical Research Fund, and the Eugene Higgins Trust Fund of Harvard University.

† Postdoctoral Research Fellow of the National Heart Institute.

1. J. Erwin and K. Bloch, unpublished.

converted into methyl esters and separated into saturated and unsaturated fractions (Goldfine and Bloch, 1961). The methyl esters of the unsaturated acids were further fractioned on silicic acid, in the form of their mercuric acetate adducts into mono-, di-, tri-, and polyenes. Analysis of the fatty acid methyl esters by vapor-phase chromatography was performed as described earlier (Goldfine and Bloch, 1961), and the individual acids identified by comparison of their retention times with those of known standards. α- and γ-Linolenic acids can be distinguished by their retention times which under the present experimental conditions were 1.86 and 1.63 respectively, relative to methyl stearate. The relative concentration of each acid was calculated from the relative peak sizes. The chain-length of unsaturated acids was determined by catalytic reduction of the isolated fractions followed by vapor-phase chromatography of the reduced products.

E. gracilis Z when grown in the light contained large amounts of α-linolenate but very little if any of this acid was present when the organism was cultured in the dark (Fig. 1). A concomitant increase in the relative concentration of a C_{20} trienoic acid and a group of unsaturated C_{22} and C_{24} acids was the only other marked difference consistently observed between cells grown in the dark and illuminated cells. When dark-grown cells were exposed to light for varying periods of time prior to harvesting, the relative concentration of α-linolenate rose steadily, increasing from 0.6% at zero time to 7.8% after 28 hours of light exposure. That these changes are not due to light per se is indicated by analysis of a number of chlorophyll-less "mutants" of Euglena.[2] The data in Table I show that the parent strain grown in the light contains large amounts of α-linolenate whereas the colorless mutants grown under identical conditions are virtually devoid of α-linolenate.

Chlorophyll synthesis and chloroplast formation in Euglena gracilis are "adaptive" phenomena occurring in response to light (Wolken, 1961; Epstein and Schiff, 1962). Dark-grown cells do not contain chloroplasts but contain instead small empty vesicles known as "proplastids." When dark-grown cells are exposed to light, these bodies mature into normal, functional chloroplasts (Epstein and Schiff, 1962). The four colorless Euglena mutants analyzed in the present study are devoid of chloroplasts,

2. Personal communication from Dr. J. Schiff

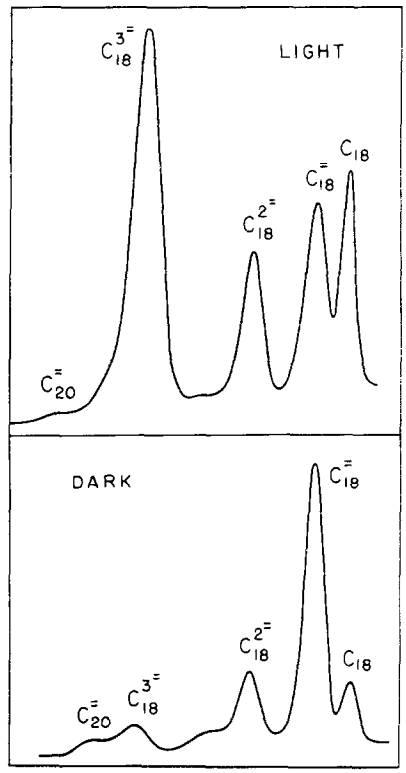

Figure 1.

Section of vapor phase chromatogram of methyl esters showing the relative amounts of C_{18} acids in the lipids of E. gracilis Z grown in the light and in the dark respectively.

---

whether grown in the light or in the dark[2] and as the present study shows, none of them contain α-linolenate in significant amounts.

In C. reinhardi, chlorophyll synthesis and chloroplast formation are constitutive, since these processes occur in the dark as well as in the light (Levine and Ebersold, 1960). The y2 mutant of this organism becomes chlorophyll-deficient when it is cultured in the dark, and it has been shown that the single large chloroplast characteristic of the species then contains a reduced number of lamellae in the internal structure (Levine and Ebersold, 1960). Analyzing the mutant and the wild type of this organism, we have found no differences in the fatty acid spectra (Table I), when both are grown in the light. However, a marked change occurs when the y2

2. Personal communication from Dr. J. Schiff

TABLE I

Relative Fatty Composition of "Wild Type" and Chloroplast-deficient "Mutants" of E. gracilis and C. reinhardi

	Relative Amounts of Fatty Acid (% of total)								
	E. gracilis Z (light)			E. gracilis var. bacillaris (light)			C. reinhardi	C. reinhardi	y2 mutant
	wild type	W_1ZXL[a]	W_2ZSL[b]	wild type	W_3BUL[c]	W_8BHL[d]	wild type light	light	dark
myristic acid	7.4	11.8	13.0	12.8	2.3	3.0	0.6	0.9	1.0
palmitic acid	14.7	23.5	29.2	14.2	23.1	8.8	24.2	21.6	35.4
palmitoleic acid	5.9	3.1	4.0	4.0	3.3	3.3	3.3	2.0	3.2
stearic acid	6.7	0.8	1.3	7.4	1.1	0.8	1.6	2.4	2.3
oleic acid	7.4	6.6	7.2	7.5	9.1	5.4	23.7	26.5	20.5
linoleic acid	5.0	1.4	3.1	2.6	2.0	1.5	4.6	4.3	5.9
α-linolenic acid	21.4	1.6	trace	15.8	1.1	0.7	31.1	27.7	9.2
eicosadienoic acid	2.4	3.3	3.9	1.7	0	0	--	--	--
eicosatrienoic acid	5.0	7.3	13.2	1.9	15.6	17.0	--	--	--
eicosatetraenoic acid	4.0	3.6	4.8	2.1	9.3	6.2	--	--	--
C_{22} and C_{24} acids[e]	7.8	21.6	12.5	10.0	17.5	41.7	--	--	--

[a] obtained by X-irradiation of the Z strain[2]
[b] spontaneous "mutant" of the Z strain[2]
[c] obtained by ultraviolet irradiation of bacillaris strain[2]
[d] obtained by heat treatment of bacillaris strain[2]
[e] unsaturated acids yielding saturated C_{22} and C_{24} acids after reduction but not further identified

mutant is grown in the dark. As in the case of E. gracilis, there is a sharp decline in the α-linolenate content, from 27.7% in the illuminated cells to 9.2% in the cells grown in the dark. (Table I).

The intracellular distribution of α-linolenate in wild type E. gracilis Z has been determined by separating chloroplast, mitochondrial, microsomal, and final supernatant fractions by published procedures (Brawerman et al, 1962). On measuring both the total fatty acid content and the composition of each fraction, it was found that 85% of the total α-linolenate in the cell was present in the chloroplast fraction. This is a minimal figure, since there was some disruption of chloroplasts resulting in contamination of other cell fractions by chloroplast fragments.

The results reported here suggest a close association between α-linolenate and the presence of functional chloroplasts in the phytoflagellates under investigation. That this may be universally true for photosynthetic organisms (except photosynthetic bacteria) is suggested by the invariable occurence of α-linolenate in the leaves but not in the stems and roots of higher plants (Hilditch, 1956; Shorland, 1962). Chloroplasts of higher plants are generally rich in α-linolenate (Zill and Harmon, 1962; Debuch, 1961), and it has also been reported that this fatty acid appears and gradually rises in seedlings of Citrullus vulgaris when cotyledons develop into first leaves (Crombie and Comber, 1956).

In view of the complete absence of polyunsaturated fatty acids in some photosynthetic bacteria (Scheuerbrandt and Bloch, 1962), it would appear that the presence of α-linolenate is a characteristic constituent of only those organisms in which photosynthesis is of the type found in green plants.

Acknowledgements

The authors wish to thank Professor R. P. Levine, Biological Laboratories, Harvard University, for providing us with the cells of C. reinhardi wild type and of the y2 mutant; Drs. J. Schiff and H. T. Epstein, Brandeis University, for cultures of the chloroplast-deficient strains of E. gracilis and Dr. L. Provasoli of Haskins Laboratories for a culture of "wild type" E. gracilis var. bacillaris.

REFERENCES

Brawerman, G., Pogo, A. O. and Chargaff, E., Biochim. Biophys. Acta, 53, 326 (1962).

Crombie, W. M. and Comber, R., J. Experimental Botany, 7, 166 (1956).

Debuch, H., Z. Naturforsch, 16 b, 246 (1961).

Epstein, H. T. and Schiff, J. A. J. Protozool., 8, 427 (1962).

Goldfine, H. and Bloch, K., J. Biol Chem., 236, 2596 (1961).

Hilditch, T. P., The Chemical Constitution of Natural Fats, John Wiley & Sons, Inc., New York, 1956, p. 146.

Hutner, S. H., Bach, M. K. and Ross, G. I. M., J. Protozool., 3, 101 (1956).

Levine R. P. and Ebersold, W. T., Z. Vererbungslehre, 89, 631 (1958).

Levine, R. P. and Ebersold, W. T., Ann Rev. Microbiol., 14, 197 (1960).

Mead, J. F., in Lipide Metabolism, (Bloch, K. Editor), John Wiley & Sons, Inc., New York, 1960, p. 41.

Riley, J. P. J. Chem. Soc., 2728 (1949).

Scheuerbrandt, G. and Bloch, J. Biol. Chem. 237, 2064 (1962).

Shorland, F. B., in Comparative Biochemistry, Vol. III, (Florkin, M. and Mason, H. S., Editors), Academic Press, New York, 1962, p. 1

Wolken, J. J., Euglena: An Experimental Organism for Biochemical and Biophysical Studies, Rutgers University Press, New Brunswick, 1961, p. 5

Zill, L. P. and Harmon, E. A., Biochim. Biophys. Acta, 53, 579 (1962).