Arachidonic Acid in the Lipids of Marine Algae Maintained under Blue, White and Red Light

Samir S. Radwan, Adel-Salam Shaaban, and Hassan M. Gebreel

Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt

Z. Naturforsch. **43c**, 15–18 (1988); received March 26/September 18, 1987

Arachidonic Acid, Light Quality, Marine Algae, Prostaglandins

Marine algae, maintained for one month under blue, white and red light were rather rich in lipids but obviously poor in fat (triacylglycerols). These lipids consisted predominantly of glycolipids and phospholipids. Irrespective of the light quality, the major constituent fatty acids in lipids of these algae were, in most cases, those with 20 carbon atoms. The light quality had a definite effect on the proportion of arachidonic acid in the lipids of certain algae. Thus, the proportion of arachidonic acid in *Enteromorpha intestinalis* maintained under white light was 45% and in *Sargassum salicifolium* kept under red light 25% of the total constituent fatty acids in the total algal lipids.

Introduction

Marine algae are sources of valuable natural products such as alginic acid, mannitol, laminarin [1], agar [2] etc. The role of phytoplankton in solving problems of pollution, energy replacement and protein shortage in the future has been reviewed [3].

Earlier lipidologists often reported that marine algae are rich in "fat", but in fact, such reports have been concerned with "total lipids" rather than with "fat". Unlike the lipids from fresh water algae those from marine algae contain relatively high proportions of polyenoic fatty acids with 20 carbon atoms or more [4–8]. Arachidonic acid (C 20:4), one of these polyenoic acids, has become of industrial value because it is used as a precursor in the synthetic production of prostaglandins [9, 10]. So far, arachidonic acid is obtained mainly from animals [10]; its production from common marine algae is certainly recommended.

The main objective of the present communication is to study whether or not higher levels of arachidonic acid can be produced in the lipids of certain common marine algae maintained continuously under specific light qualities.

Materials and Methods

Four algae belonging to Chlorophycophyta namely *Ulva lactuca* L., *Enteromorpha intestinalis* (L.) Link., *Codium tomentosum* f. *tomentosum* (Hudson) Stack and *Codium tomentosum* f. *divericatum* Ag., as well as one belonging to Rhodophycophyta. *Gelidium latifolium* Born. and one to Phaeophycophyta, *Sargassum salicifolium* (Bert.) J. Ag. were collected from the Mediterranean Sea at the coast of Alexandria, Egypt. The algae were maintained in sea-water in glass jars that were wrapped with blue (transmitting maximum 520 nm waves), colourless or red (730 nm) cellophane paper purchased from the local company "Racta". The jars were incubated for one month at 25 °C in an illumination chamber, 1 m^3, provided with 6 fluorescent lamps, 40 watt each. The sea-water was changed in intervals of 2 days.

Lipids were extracted and purified following established methods [11, 12]. The classes of nonpolar lipids were separated by TLC on silica gel with hexane–ether–acetic acid (90:10:1) [13]. The classes of polar lipids were resolved by two-dimensional TLC with chloroform–methanol–7 N NH_4OH (65:30:4) in the first direction and chloroform–methanol–acetic acid–H_2O (170:25:25:6) in the second direction [11]. Lipid classes were identified by comparing their chromatographic behaviour with that of reference samples and by their reactions with specific spray reagents [14, 15]. Quantitative analyses were done by determining the constituent fatty acids in TLC fractions by GLC using 17:0 as an internal standard [16].

Aliquots of the total lipids and fractions resolved by TLC were subjected to methanolysis [17]. The resulting methyl esters were purified by TLC [13] and analyzed by GLC on a polar (15% DEGS on Anakrom D) and nonpolar column (10% Silar 5 CP on Gas-Chrom Q).

Reprint requests to Prof. S. S. Radwan, Head of the Department of Botany and Microbiology, Faculty of Science, Kuwait University, P. O. Box 5969, 13060 Safat Kuwait.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/88/0100–0015 $ 01.30/0

Results

Lipid contents

The total lipid contents of various marine algae that, after their collection, were maintained for one month in sea-water under continuous illumination with blue, white and red light are presented in Table I.

It is apparent that the total lipid contents varied, not only according to the alga studied but also, for the same alga, with changing the light quality.

Composition of lipid classes

The TLC analyses showed that in all lipid extracts of marine algae maintained under white light the ionic and other polar lipids predominated over the nonpolar lipids. The polar lipid classes consisted of mixtures of phospholipids and glycolipids. The major phospholipids in all extracts were diacylglycerophosphoglycerols and diacylglycerophosphoethanolamines. In the extracts of the green alga *Ulva lactuca* only relatively high proportions of diacylglycerophosphoinositols were also observed. Low proportions of other unidentified phospholipids occurred in the various extracts. In the lipid extracts from the different marine algae the levels of diacylglycerophosphocholines were very low. The glycolipids consisted of monogalactosyldiacylglycerols (MGDG) and digalactosyldiacylglycerols (DGDG) with the former class obviously predominating over the latter. The quantitative analyses of these two classes in the lipid extracts from *Ulva lactuca* showed that MGDG and DGDG made-up 15.1 and 3.0% of the total lipids, respectively. The nonpolar lipids in the various extracts consisted of triacylglycerols, sterols and hydrocarbons. The proportions of triacylglycerols were low.

Table I. Lipid contents (% of dry wt.) of algae maintained under different light colours.

Algae	Red light	White light	Blue light
Green algae:			
Ulva lactuca	6.9	9.8	8.2
Enteromorpha intestinalis	11.5	11.5	16.9
Codium tomentosum f. *tomentosum*	27.4	10.6	28.2
Codium tomentosum f. *divericatum*	34.7	17.4	31.3
Red alga:			
Gelidium latifolium	11.6	14.9	37.8
Brown alga:			
Sargassum salicifolium	4.9	7.1	4.7

The TLC analyses did not show any obvious variation in the levels of the various lipid classes, with the exception of the glycolipids, in response to changing the light quality. As a rule, the proportions of MGDG in the lipids from algae maintained under white light were higher than their proportions from algae kept under blue or red light. The quantitative analyses showed that the ratio MGDG/DGDG in the lipids from *Ulva lactuca* decreased from about 5.0 under white light to about 0.5 and 1.0 under blue and red light, respectively.

Fatty acid patterns of total lipids

The total lipids from marine algae maintained under different light colours contained fatty acids with even carbon chains ranging in length between 14 and more than 20 carbon atoms. Irrespective of the light quality, the major fatty acids in the extracts from the green algae studied were those with 20 carbon atoms (Fig. 1).

The C 20 fatty acids also predominated in the lipids from the red alga *Gelidium latifolium* but only from the samples that have been maintained under white light. Under blue and red light the major fatty acids in the lipids from this red alga were those with 16 carbon atoms. The lipids from the brown alga *Sargassum salicifolium* maintained under various light colours contained almost equal proportions of the fatty acids with 14, 16, 18 and 20 carbon atoms.

The data in Table II show that, in most cases, the lipids from the various marine algae contained, as a major saturated fatty acid that with 20 carbon atoms (arachidic acid). The lipids from the red alga *Gelidium latifolium* kept under blue or red light contained palmitic acid (C 16:0) and those from the brown alga *Sargassum salicifolium* maintained under red light contained myristic acid (C 14:0) as the predominant saturated fatty acids.

In the various extracts there were different proportions of unsaturated C 14, C 16 and C 18 fatty acids with up to 3 double bonds in addition to unsaturated C 20 acids with up to 5 double bonds. There were no common correlations between the proportions of these unsaturated fatty acids in the lipids of various algae and the light quality under which these algae have been maintained. However, certain algae main-

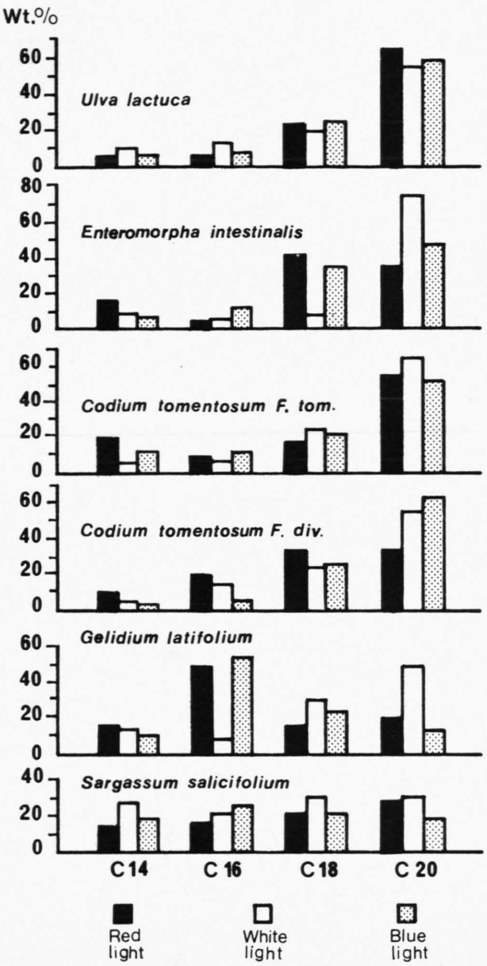

Fig. 1. Constituent fatty acids of total lipids from marine algae separated according to the chain length of these acids.

tained under specific light qualities contained in their lipids high proportions of arachidonic acid (C 20:4). Thus, the green alga *Enteromorpha intestinalis* kept under white light and the brown alga *Sargassum salicifolium* maintained under red light contained in their lipids 44.7 and 24.9% of arachidonic acid, respectively.

Discussion

The study of the lipids from marine algae is important both for basic science as well as from the industrial view-point. Algae of various colours are suitable materials in basic studies concerned for example with investigating the role of certain lipid classes in photosynthesis [8]. From the industrial view-point, marine algae, being of wide distribution may represent suitable sources of valuable lipids and/or fatty acids.

Our results show that although marine algae contain considerable amounts of lipids, their "fat" contents are rather low. It appears that these organisms possess only a limited capacity to accumulate triacylglycerols.

Resembling other photosynthetically active plants and plant organs, marine algae contain in their total lipid extracts mainly phospholipids and glycolipids. Confirming earlier findings [18, 19] the levels of diacylglycerophosphocholines are very low but the proportions of diacylglycerophosphoglycerols, the only phospholipid in the chloroplasts [8], and diacylglycerophosphoethanolamines are relatively large. This information is also of interest from the phylogenetic point of view because it is known that Cyanobacteria synthesize as a major phospholipid only diacylglycerophosphoglycerols [20]. The two classes of glycolipids always found in the lipids from photosynthetically active plants and plant organs, namely MGDG and DGDG, are also found in the lipids from marine algae. As in higher plants [21] and isolated chloroplasts [9, 22, 23] the proportions of MGDG are higher than those of DGDG in the lipids from marine algae maintained under white light but not from those kept under blue or red light.

It appears that there is no common correlation between the light quality and the lipid contents of various marine algae. Yet, the two varieties of the green alga *Codium tomentosum* studied accumulated more lipids under blue and red light than under white light. Also the red alga *Gelidium latifolium* produced more lipids under blue light than under white or red light. These results were basically confirmed in several analyses.

An interesting observation is that arachidic acid (20:0) is the major saturated fatty acid in the lipids of several marine algae. It is well-known that palmitic acid (16:0) is the predominant saturated fatty acid in the lipids from fresh water algae and higher plants. It thus, appears that marine algae are among the most suitable plants for obtaining fatty acids with higher chain length.

The levels of arachidonic acid (20:4) in the lipids of several marine algae exposed to specific light qualities are especially high. Thus, for example 45% of

Table II. Constituent fatty acids of total lipids from various marine algae⁺.

Fatty acids	Green Algae												Red alga			Brown alga		
	Ulva lactuca			*Enteromorpha intestinalis*			*Codium tomentosum* f. *tomentosum*			*Codium tomentosum* f. *divericatum*			*Gelidium latifolium*			*Sargassum salicifolium*		
	Red light	White light	Blue light	Red light	White light	Blue light	Red light	White light	Blue light	Red light	White light	Blue light	Red light	White light	Blue light	Red light	White light	Blue light
14:0	3.7	1.9	4.2	7.4	2.6	2.1	9.1	1.6	6.5	4.2	1.7	1.5	11.8	6.7	4.1	13.1	17.5	7.7
14:1	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
14:2	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
14:3	1.8	7.4	2.0	7.4	5.3	4.0	9.1	3.4	4.3	5.7	0.7	0.6	3.5	6.7	5.7	tr.	10.7	11.4
16:0	2.4	2.1	2.1	1.4	0.1	0.5	4.3	0.6	6.0	11.7	4.2	2.6	36.1	tr.	24.8	tr.	3.5	6.8
16:1	1.2	2.0	1.7	3.6	3.3	0.5	3.1	0.9	3.3	tr.	6.2	2.6	11.8	6.7	29.1	7.5	2.9	4.4
16:2	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	8.6	tr.	tr.
16:3	2.0	6.6	1.5	tr.	1.1	9.6	tr.	4.6	2.9	7.4	4.8	tr.	tr.	tr.	tr.	7.7	15.3	5.1
18:0	4.5	6.0	7.9	6.7	3.0	3.5	2.7	9.9	12.5	7.9	12.8	6.4	3.5	14.6	4.3	3.3	7.3	7.2
18:1	2.8	3.7	2.3	18.5	2.9	5.8	2.7	2.5	4.7	21.8	6.4	3.0	8.1	7.4	8.5	tr.	1.3	2.0
18:2	5.1	3.7	5.1	18.0	5.4	25.2	11.5	7.5	5.0	2.9	7.2	5.2	4.0	7.8	4.1	14.6	7.9	6.5
18:3	10.6	8.2	10.0	tr.	tr.	tr.	tr.	2.9	tr.	2.8	tr.	12.5	0.3	tr.	5.3	5.4	tr.	tr.
20:0	25.1	16.5	21.2	19.8	19.9	23.3	12.5	34.1	18.9	4.0	19.5	2.0	1.9	14.5	0.8	tr.	22.8	13.2
20:1	10.5	7.2	12.5	5.4	4.8	3.8	14.5	14.5	14.8	1.2	2.2	39.7	3.8	tr.	6.3	tr.	7.3	4.1
20:2	0.6	1.3	0.8	4.7	5.3	4.9	14.3	0.5	tr.	1.4	3.1	tr.	tr.	tr.	tr.	8.0	1.6	tr.
20:3	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
20:4	13.4	9.6	16.7	4.5	44.7	11.6	7.7	10.9	5.0	3.0	10.6	6.1	3.8	10.1	4.9	24.9	1.9	2.9
20:5	13.2	10.7	8.8	2.7	1.6	5.3	tr.	3.4	tr.	6.2	tr.	1.0	11.4	24.6	1.9	tr.	tr.	tr.
>20	3.0	13.2	3.0	tr.	tr.	tr.	8.6	3.0	15.5	20.0	20.6	16.8	tr.	tr.	tr.	tr.	tr.	tr.

⁺ Data are expressed as wt. %.

arachidonic acid is produced by *Enteromorpha intestinalis* exposed continuously to white light. In this context, it is to be noted that marine algae in their natural environments occur at various depthes and are consequently exposed to various light qualities throughout the day only.

Our results show clearly that it is possible to enrich certain marine algae with arachidonic acid by keeping them under continuous illumination with light of particular wave-length. This provides a suitable economic source for this medicinally interesting fatty acid.

[1] A. F. Abdel-Fattah and M. M. Hussein, Phytochemistry **9,** 721 (1970).

[2] J. N. C. Whyte and J. R. Englar, Phytochemistry **20,** 237 (1981).

[3] F. Sauze, Tech. Sci. Munic. **12,** 537 (1975).

[4] E. Klenk, W. Knipprath, D. Eberhagen, and H. P. Koof, Hoppe-Seyler's Z. Physiol. Chem. **334,** 44 (1963).

[5] H. Wagner and P. Pohl, Biochem. Z. **341,** 476 (1965).

[6] S. Patton, G. Füller, A. R. Loeblich, and A. A. Benson, Biochim. Biophys. Acta **116,** 577 (1966).

[7] B. W. Nichols and R. S. Appleby, Phytochemistry **8,** 1907 (1969).

[8] P. J. Quinn and W. P. Williams, Biochim. Biophys. Acta **737,** 223 (1983).

[9] C. Pace-Asciak and L. S. Wolfe, Biochemistry **10,** 3657 (1971).

[10] E. W. Horton, Prostaglandins, Springer Verlag, Berlin 1972.

[11] B. W. Nichols, New Biochemical Separations (A. T. James and L. J. Morris, eds.), p. 321, van Nostrand, London 1964.

[12] J. Folch, M. Lees, and G. H. Sloane-Stanley, J. Biol. Chem. **226,** 497 (1957).

[13] H. K. Mangold and D. C. Malins, J. Am. Oil Chem. Soc. **37,** 383 (1960).

[14] J. C. Dittmer and R. L. Lester, J. Lipid Res. **5,** 126 (1964).

[15] A. N. Siakotos and G. Rouser, J. Am. Oil. Chem. Soc. **42,** 913 (1965).

[16] S. S. Radwan, J. Chromatogr. Sc. **16,** 538 (1978).

[17] A. Chalvardijian, Biochem. J. **90,** 518 (1964).

[18] M. G. Macfarlane and L. W. Wheeldon, Nature **183,** 1808 (1959).

[19] J. F. G. M. Wintermans, Biochim. Biophys. Acta **44,** 49 (1960).

[20] B. W. Nichols, R. V. Harris, and A. T. James, Biochim. Biophys. Res. Commun. **20,** 256 (1965).

[21] P. G. Roughan and R. D. Batt, Phytochemistry **8,** 363 (1969).

[22] C. Hitchcock and B. W. Nichols, Plant Lipid Biochemistry, Academic Press, London 1971.

[23] S. S. Radwan and H. K. Mangold, Adv. Biochem. Engin., p. 16, Springer Verlag, Berlin 1980.