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ALTERATIONS IN LIPID METABOLISM CAUSED BY ILLUMINATION OF THE MARINE RED ALGAE *CHONDRUS CRISPUS* AND *POLYSIPHONIA LANOSA*

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Key Word Index—*Chondrus crispus*; *Polysiphonia lanosa*; Rhodophyceae; red algae; lipid metabolism; fatty acids; light, effect of.

Abstract—The effect of illumination on lipid metabolism has been studied in the two red marine algae, *Chondrus crispus* and *Polysiphonia lanosa*. Light stimulated the incorporation of radiolabel from [^{14}C]acetate into monoglycosyldiacylglycerol, diglycosyldiacylglycerol and phosphatidylglycerol in *C. crispus*. Few changes were seen in *P lanosa*. Phosphatidylglycerol was the most rapidly labelled lipid in the light in both algae. At early incubation times it had the highest specific radioactivity—possibly indicating a major role in fatty acid metabolism, as suggested for green algae. Although the fatty acid labelling patterns for the two algae were different, illumination stimulated the labelling of oleic and linoleic acid in both species.

INTRODUCTION

Little is known about lipid content and lipid metabolism in marine algae and how these are affected by environmental factors. This is in spite of the fact that these organisms constitute a large part of the Plant Kingdom. Pohl and Zurheide [1] summarized the literature up to 1979 on the fatty acids and lipids of marine algae and the influence of the environment on their endogenous levels. Changes in major environmental conditions such as light and temperature are known to cause changes in composition and metabolism of lipids in higher terrestrial plants [2] and in the few macro-algae [1, 3–7] and unicellular algae [8, 9] which have been studied.

An effect of illumination is generally to increase fatty acid synthesis and desaturation, probably by increasing the rate of enzyme synthesis and/or supply of co-factors needed for these processes [10, 11]. Also, light often causes an increase in synthesis of those lipids considered typical of the chloroplasts (i.e. PG, MGDG, DGDG, and SQDG [2]) while the relative rate of synthesis for those lipids which have a largely extra-chloroplastic location (i.e. PC, PE and neutral lipids) declines. However, whereas higher plants show a very large increase in fatty acid synthesis and acyl lipid formation in the light, the marine brown alga, *Fucus serratus* was less affected [3, 4]. Moreover, work with subcellular fractions from the marine alga, *Bryopsis maxima* showed that light stimulation of fatty acid synthesis was confined to the chloroplast [12].

As part of a study on the lipid biochemistry of two intertidal marine red algae, *Chondrus crispus* and *Polysiphonia lanosa*, an examination of some of the effects of illumination was carried out to see how well their lipid metabolism corresponded to that in higher plants and other algae. Such studies were considered particularly interesting because of the special structures of red algal photosynthetic membranes and because the effects of illumination on their membrane lipid synthesis has not been studied previously. A preliminary report of some of this data has been made [13].

RESULTS AND DISCUSSION

For photosynthetic organisms light availability is of prime importance and thus can be expected to have a number of effects on plant lipid metabolism. However, it should be noted that like many environmental factors, illumination has several facets such as light quality, light intensity and photo-period, each of which can interact to affect metabolism and whose effects are difficult to separate. Bright light can also cause heating and/or dessication of the system under study, adding yet more complications to hinder interpretation. Hence all conclusions must be drawn very carefully.

In this first study, the effect of illumination on the lipid metabolism of *C. crispus* and *P. lanosa* was examined in the simplest way in order to see if any large changes could be observed. To do this, the incorporation and distribution of radiolabel from [^{14}C]acetate, in the fatty acids and lipids, was measured in the presence and absence of light.

The effects of illumination on the uptake and distribution of radiolabel in *C. crispus* is shown in Table 1. Illumination stimulated total [^{14}C]acetate uptake two- to

Abbreviations: MGDG, monoglycosyldiacylglycerol (mainly monogalactosyldiacylglycerol [25]); DGDG, diglycosyldiacylglycerol (mainly digalactosyldiacylglycerol [25]); SQDG, sulphoquinovosyldiacylglycerol; PC, phosphatidylcholine (this band also includes some phosphatidylsulphocholine (PSC) when separated by TLC [25]); PE, phosphatidylethanolamine; PG, phosphatidylglycerol; TAG, triacylglycerol; FFA, free (unesterified) fatty acid.

Table 1. Effects of illumination on the uptake and distribution of radiolabel from [^{14}C]acetate in *C. crispus*

Incubation time (hr)		Total uptake	% of counts recovered: Aqueous phase	Organic phase (O)	% O label in acyl lipids (L)	% L label in fatty acids
1	(D)	2.1 ± 0.7	50 ± 20	50 ± 17	30 ± 16	90 ± 12
	(L)	4.6 ± 1.3	20 ± 3	80 ± 19	7 ± 1	50 ± 5
8	(D)	4.5 ± 0.3	32 ± 10	68 ± 33	44 ± 12	86 ± 33
	(L)	10.4 ± 1.0	49 ± 15	51 ± 9	20 ± 2	68 ± 16
24	(D)	5.8 ± 0.2	26 ± 11	74 ± 24	64 ± 10	96 ± 2
	(L)	11.3 ± 0.2	38 ± 2	62 ± 7	19 ± 2	60 ± 5

Values are mean ± s.d. ($n = 3$) for material collected in October. Incubation temperature = 15°. Total uptake in dpm/g fresh weight ($\times 10^{-6}$).
(D) = incubated in dark, (L) = incubated in light.

three-fold, although there was often quite a wide variation in uptake between the individual samples within a single treatment group. The rate of uptake in *C. crispus* was about half that in *P. lanosa* (data not shown), indicating a more active transport process in the latter. Again, light stimulated total [^{14}C]acetate uptake in *P. lanosa*.

The relative amount of labelling of the acyl lipids, as a percentage of total lipids, was decreased in the light compared to the dark for *C. crispus* (Table 1). However, when taking into account the greater uptake of radioactivity, it will be seen that the labelling of total acyl lipids in *C. crispus* was little affected by light exposure. In contrast, it was the labelling of the pigment fraction from [^{14}C]acetate which increased markedly. Additionally, whereas most (> 86%) of the total radiolabel in *C. crispus* acyl lipids was found in the acyl chains at all incubation times in the dark, only 60% was found there after 24 hr in the light. This, in part, may have reflected the increased labelling of glycosylglycerides whose carbohydrate moieties are well labelled from [^{14}C]acetate in many plants [see 14]. In addition, after 24 hr about 50% of the total label remained insoluble (data not shown), probably due to incorporation into insoluble cell components such as membrane-bound proteins and cell-wall constituents. This was found for both light and dark incubations.

In spite of having little affect on the total labelling of acyl lipids in *C. crispus*, illumination was found to alter significantly the proportion of radiolabel in different lipid classes (Table 2). Thus, illumination tended to increase the relative labelling of MGDG, DGDG and PG while decreasing that for PC, TAG and non-esterified fatty acids. The relative labelling of free sterol from *C. crispus* also decreased (data not shown).

The increased labelling of *C. crispus* PG, MGDG and DGDG in the light agrees well with previous studies on the green algae *Chlorella vulgaris* and *Euglena gracilis* [8] although similar studies on the marine brown alga *F. serratus* [3] found no significant changes in the glycolipids and only a small increase in the relative labelling of PG. The glycosylglycerides and PG are largely confined to the chloroplast membranes in higher plants and green algae and, therefore, it might be expected that illumination would influence their radiolabelling. In particular it has been shown that high rates of labelling of these lipids can be obtained in tissues where chloroplasts are developing [e.g. 15]. PC and the neutral lipids are largely extrachloroplastic in plants [16] and, assuming that such a distribution holds for the red algae, their synthesis would be expected to be less dependent on light availability.

Fewer consistent or obvious changes were seen in *P. lanosa* (Table 3), possibly because of damage caused when excising the epiphyte from its host *Ascophyllum nodosum* or, alternatively, as suggested for *F. serratus* [3], because the 'chloroplast' lipids may be present in large amounts outside the chloroplasts. These extrachloroplastic pools could dilute out any plastid-localized changes induced by light. A comparison of the data for isolated *P. lanosa* chloroplasts (unpublished results) with those for whole tissue supports this idea because there is very little difference in their respective lipid compositions.

When the specific radioactivities were calculated from the amount of radiolabel in a known amount of lipid, PG was found to rapidly attain a high specific activity in the light for both algae (see Fig. 1 for *C. crispus*), PC (+ phosphatidylsulphocholine), MGDG and TAG were also found to reach high specific radioactivities.

The rapid labelling of PG in the light suggests the possibility that it may be a primary acceptor of newly synthesized fatty acids and/or a site for an early fatty acid desaturation step before acyl transfer to other lipids. This is supported by the time-course data for *C. crispus* (Table 2) where the relative labelling of PG fell with time. Similar observations were made by Nichols *et al.* [17] with the green alga *Chlorella vulgaris* and these authors suggested that PG could be involved in fatty acid desaturation. Experiments with *Dunaliella salina* [18] have also revealed a very rapid labelling of the PG fraction. In *Fucus serratus* illumination was found to increase the radiolabelling of PG from [^{32}P]orthophosphate [5]. Moreover, a similar increase in such labelling was found in *Chlorella vulgaris* [19] indicating that its synthesis may be particularly stimulated by light. Thus, the increased ^{14}C-labelling in the red algae studied here may also be partly due to stimulation of PG synthesis as well as stimulation of acyl chain turn-over in this phospholipid. PC has also been implicated in fatty acid desaturations and in providing acyl moieties for TAG formation [20, 21]. Such functions would explain the time-course and

Table 2. The effect of illumination on the distribution of radiolabel between the acyl lipids of *C. crispus*

	Radiolabel incorporation (% ^{14}C)					
	Incubation time (hr)					
	1		2		24	
Acyl lipid	(D)	(L)	(D)	(L)	(D)	(L)
MGDG1	3.8	5.9	*3.9	8.4	10.0	11.7
	±1.3	±0.3	±2.2	±1.5	±0.3	±1.9
MGDG2	2.1	1.9	1.8	2.6	1.1	2.8
	±1.6	±0.3	0.7	±0.5	±0.2	±0.2
DGDG	*1.3	3.1	**1.3	3.0	**2.0	7.0
	±0.3	±0.9	±0.3	±0.3	±0.3	±0.5
SQDG1	4.7	6.4	5.4	5.9	6.8	8.9
	±0.5	±3.8	±2.2	±1.0	±0.8	±3.9
SQDG2	4.4	5.8	3.5	3.6	3.3	4.3
	±0.7	±2.2	±0.3	±0.8	±0.3	±1.0
PC+PSC	*31.2	19.5	**37.9	17.2	29.1	17.9
	±5.5	±1.4	±5.7	±1.6	±5.0	±2.9
PG	20.8	22.2	**14.0	24.8	**5.8	18.8
	±5.0	±0.3	±2.3	±2.1	±1.9	±1.4
TAG	3.1	3.9	8.8	5.6	**26.3	5.3
	±1.6	±1.2	±5.7	±2.8	±1.6	±0.1
FFA	5.3	2.7	6.6	3.7	**3.5	1.0
	±1.9	±1.1	±1.9	±1.0	±0.2	±0.1
Others	23.3	28.4	16.8	25.2	12.1	22.3
	±1.9	±5.2	±2.9	±3.1	±3.2	±4.2

Values are means ± s.d. ($n=3$) for material collected in October. Incubation temperature = 15°. (D) = incubated in dark; (L) = incubated in light. Statistical analysis was carried out using Student's t test; $^{*}P<0.05$, $^{**}P<0.01$.

Other lipids include phosphatidylethanolamine, phosphatidic acid, diphosphatidylglycerol, sterol esters, two unknown lipids and monoacyl derivatives of identified lipids. MGDG and SQDG both separated into two bands on TLC with the slower moving band designated 1 [see 25].

Table 3. The effect of illumination on the distribution of radiolabel between the acyl lipids of *P. lanosa*

	Radiolabel incorporation (% ^{14}C)					
	Incubation time (hr)					
	1		2		24	
Acyl lipid	(D)	(L)	(D)	(L)	(D)	(L)
MGDG	28.4	24.9	29.3	30.0	23.0	22.7
	±8.1	±6.1	±4.5	±6.2	±6.4	±1.7
SQDG	10.1	6.6	8.3	7.0	9.0	7.2
	±1.6	±1.9	±2.3	±0.5	±2.7	±1.7
DGDG	5.2	2.7	5.6	3.9	11.5	9.9
	±1.7	±0.3	±1.7	±0.7	±4.8	±2.8
PC+PSC	14.3	10.5	8.5	8.6	9.5	11.6
	±4.0	±1.0	±1.0	±1.4	±1.5	±3.1
PG	**15.6	32.2	28.0	29.1	32.6	24.1
	±0.7	±5.2	±11.3	±8.3	±17.0	±4.3
TAG	**3.2	0.6	*2.2	0.7	2.7	1.7
	±1.2	±0.1	±0.5	±0.3	±0.9	±1.0
FFA	2.7	2.2	2.1	1.4	1.3	1.7
	±0.2	±0.5	±0.5	±0.3	±0.1	±0.8
Others	20.5	20.3	16.0	19.3	10.4	21.1
	±5.5	±3.6	±2.1	±2.6	±4.3	±5.9

Values are means ± s.d. ($n=3$) for material collected in November. See Table 2 for explanations.

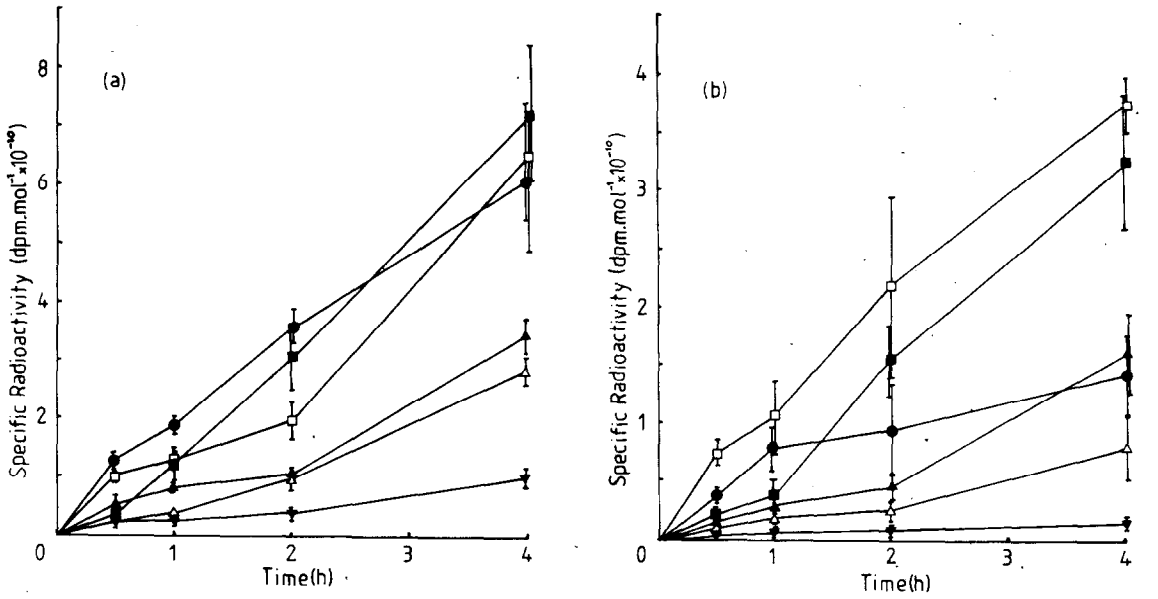

Fig. 1. The effect of illumination on the specific radioactivities of the major acyl lipids from *C. crispus*. The figure shows the specific radioactivities (±s.d. where $n=3$) for *C. crispus* with (a) and without (b) illumination. ●, PG; □, PC(+PSC); △, MGDG; ▼, DGDG; ▲, SQDG; ■, TAG. The different molecular species of MGDG, DGDG and SQDG have been combined in this figure.

Table 4. Effects of incubation time and illumination on the distribution of radiolabel between the fatty acids of *C. crispus*

Incubation time (hr)		% radiolabel in fatty acids						
		14:0	16:0	16:1	18:0	18:1	18:2	Others
1	(D)	tr	15±3	3±2	*10±3	66±10	3±3	3±1
	(L)	tr	10±2	tr	2±1	79±8	8±7	1±1
2	(D)	tr	16±2	1±1	11±6	*60±5	3±3	tr
	(L)	tr	14±2	tr	7±2	72±3	7±2	tr
8	(D)	tr	24±9	7±3	**11±6	**44±2	6±2	**8±3
	(L)	tr	11±2	3±2	tr	77±2	8±2	1±1
24	(D)	**6±2	**31±2	2±1	**7±3	*40±7	**3±1	*11±2
	(L)	1±1	19±2	3±1	tr	64±7	10±2	3±3

Values are means ± s.d. ($n=3$) for material collected in October. Incubation temperature = 15°. (D) = incubated in dark, (L) = incubated in light, tr = trace (<0.5%). Significance was tested by Student's *t* test; $*P<0.05$, $**P<0.01$.

Fatty acid abbreviations: 14:0, myristate; 16:0, palmitate; 16:1, palmitoleate; 18:0, stearate; 18:1, oleate; 18:2, linoleate.

the relative specific activities of the data in Fig. 1, especially in the dark where the contribution of PG could be expected to be less important.

In both *C. crispus* and *P. lanosa*, the percentage of label in oleic and linoleic acid increased upon illumination while that for myristic, palmitic and stearic acid decreased (Tables 4 and 5). Only trace amounts of label were detected in the C_{20} polyunsaturated acids within the 24 hr period of the incubations, indicating a slow rate of synthesis and turn-over for these quantitatively very important acids.

The reasons for the increased synthesis and desaturation of fatty acids in the light are not entirely clear. However, photosynthesis probably causes these changes by increasing the need for thylakoid membrane replacement, by raising the amounts of desaturase enzymes or, more likely, by increasing the supply of co-factors needed for these processes. Evidence for these changes being related to increased supply of co-factors comes from the fact that the products of photosynthesis such as oxygen and reduced ferredoxin (a by-product of photosynthesis) have been shown to be necessary for stearate desaturation [10, 11]. Moreover, removal of stearate by desaturation should reduce end-product inhibition from condensing enzyme 2 of the fatty acid synthetase [see 16] and, therefore, increase the ratio of C18/C16 products. An

Table 5. Effects of incubation time and illumination on the distribution of radiolabel between the fatty acids of *P. lanosa*

Incubation time (hr)		% radiolabel in fatty acids						
		14:0	16:0	16:1	18:0	18:1	18:2	Others
1	(D)	4±2	**29±2	9±2	5±1	*42±7	10±2	1±tr
	(L)	1±tr	20±3	6±2	3±1	57±1	13±2	tr
2	(D)	5±1	30±5	9±2	11±3	38±9	6±2	1±tr
	(L)	3±1	26±5	6±1	5±3	49±11	9±2	2±1
8	(D)	*12±3	32±6	10±2	10±2	23±3	10±2	3±1
	(L)	3±2	38±11	5±3	7±4	30±11	12±6	5±2
24	(D)	3±2	34±14	6±2	*8±3	43±16	** 4±1	*2±1
	(L)	1±tr	17±6	5±1	2±1	59±2	16±3	tr

Values are means ± s.d. ($n=3$) for material collected in November. See Table 4 for explanations.

increase in this ratio was seen for both algae in the light (Tables 4 and 5).

In *P. lanosa*, palmitate and oleate were the most highly labelled fatty acids, accounting for *ca* 70% of the radiolabel, while nearly all the rest was distributed between myristate, palmitoleate, stearate and linoleate. In *C. crispus*, most of the label was found in oleate, although the proportion decreased with time while that in palmitate increased. These patterns of fatty acid radiolabelling are similar to those obtained for other marine [3, 4] or freshwater algae [8, 17]. Moreover, in *Fucus serratus* [3, 4], *Chlorella vulgaris* and *Euglena gracilis* [8] light was also demonstrated to increase the relative labelling of unsaturated fatty acids from [^{14}C]acetate.

The present experiments have demonstrated that light not only stimulates total lipid synthesis but also changes the pattern of fatty acids and acyl lipids synthesized by marine red algae. Furthermore, many of the alterations in labelling patterns seen in *C. crispus* and *P. lanosa* are also found in freshwater algae and higher plants [2] although, noticably, whereas synthesis is significant in the dark for marine algae [3, 4] it has only low rates in higher plants.

It might be concluded that most of the changes in lipid metabolism seen on illumination are concentrated in those compounds which are important in chloroplast membranes [22]. However, the mechanisms behind many of these changes remain obscure and even contradictory. Not only must further experiments be directed towards elucidating the control mechanisms involved but consideration should also be given to questions such as the possible effect of light quality—a factor known to influence the synthesis of certain fatty acids and lipids [23, 24]. In addition, one has to determine whether the changes in lipid metabolism observed in the light represent adaptations or merely responses to environmental changes.

EXPERIMENTAL

Fatty acid standards were obtained from Nu-Check Prep. Inc. (Elysian, MN 56028, U.S.A.) and lipid standards from Sigma or isolated from leaf tissue [16]. Na[1−^{14}C]Acetate (57 μCi/μmol) was obtained from Amersham International, Amersham, U.K.

Chondrus crispus and *Polysiphonia lanosa* (an obligate epiphyte on the brown alga *Ascophyllum nodosum*) were harvested at Sully (G.R. ST167673) on the South Wales coast at low tide. Algae were kept in tanks of filtered, sterilized seawater at 4° under illumination of *ca* 200 μE/m^2 sec (warm white fluorescent tubes) with a photo-period corresponding to prevailing day length and aerated by a compressed air line. They could be kept in this way for up to 3 weeks without any noticable change in morphology or metabolism.

Isotope labelling Tissue samples of about 50 mg fr. wt. were washed in 0.5% Triton X-100 to remove surface microbial contamination [see 5], blotted dry and weighed before placing in a screw-capped test tube containing 3 ml sterile seawater. The alga was then pre-incubated for 1 hr at 15° before [1−^{14}C]acetate was added (usually 1 μCi) and the sealed tube placed in a shaking water bath at 15° for 0.5, 1, 2, 4, 8 or 24 hr in darkness or in white light (illumination at about 200 μE/m^2. sec using warm white fluorescent tubes). After incubation, an aliquot of the incubation medium was taken for scintillation counting to determine the total uptake of radiolabel. The lipids were extracted and divided into two equal fractions. One fraction was transmethylated and analysed by radio-GLC and the other was separated into the individual lipids by CC and TLC.

Radioactive samples were counted in a scintillant of PCS (Amersham International, Amersham)–xylene (2:1, v/v) using an Intertechnique SL4000 scintillation counter. Quench corrections were made using the external standard method. Radioactive fatty acids were counted using a gas flow proportional counter after catalytic oxidation to CO_2 [25].

Lipid analysis Lipid extractions were carried out by a modification of the Garbus method [26] as shown to be quantitative for red algae [25]. Methods for transmethylation and lipid separation, identification and analysis are also given in ref. [25]. Briefly, transmethylation was carried out using 2.5% H_2SO_4 in dry MeOH at 70° for 2 hr in the presence of an internal standard. GC separations were carried out routinely on an EGSS-X column after isolating the fatty acid methyl esters by TLC. Total lipids were fractionated by Florisil column chromatography before TLC separations. Identifications were based routinely on co-chromatography but degradative analyses and other identification procedures had been carried out previously on the individual fatty acids and lipids [25].

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