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LIPID METABOLISM IN THE RED MARINE ALGAE *CHONDRUS CRISPUS* AND *POLYSIPHONIA LANOSA* AS MODIFIED BY TEMPERATURE

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Key Word Index—*Chondrus crispus*; *Polysiphonia lanosa*; Rhodophyceae; red algae; lipid metabolism; fatty acids; temperature effects.

Abstract—Effects of temperature on lipid content *in vivo* and metabolism *in vitro* were studied in the two red marine algae, *Chondrus crispus* and *Polysiphonia lanosa*. The amounts of phosphatidylglycerol, triacylglycerol and non-esterified fatty acid increased during the winter in *P. lanosa*, as did the degree of unsaturation. The proportion of radiolabel from [^{14}C]-acetate in diglycosyldiacylglycerol and sulphoquinovosyldiacylglycerol *in vitro* increased with rising temperature in *C. crispus* while that for phosphatidylglycerol fell. Rising temperature also increased the proportion of radiolabelling in myristate and palmitate while reducing that for oleate in both algae. These results are discussed in terms of adaptation to changed environmental temperatures and mechanisms for the control of membrane lipid fluidity.

INTRODUCTION

Despite their prevalence, marine algae are a poorly studied group of organisms and little is known of their lipid metabolism. The limited data available indicates that the fatty acid compositions of these algae may alter in response to changing seasonal water temperatures in a similar way to that seen for temperature-induced changes in terrestrial higher plants [1, 2]. Thus, Pohl and Zurheide [3] found that the cooling of the Baltic Sea in winter led to increased fatty acid desaturation in the red alga *Phycodrys sinuosa* and the brown alga *Fucus vesiculosus*. Jamieson and Reid [4] also found increased desaturation during the winter months in several species of brown algae. Moreover, Smith and Harwood [5] found a similar response when the labelling of fatty acids from [^{14}C]-acetate was followed at different temperatures in the brown alga, *Fucus serratus* [see 6].

The effect of temperature on individual lipid classes is less clear since some plants such as rye have been found to alter their levels [7] while others do not [1]. In *Fucus serratus* [5] and *Phycodrys sinuosa* [3] no significant temperature-related changes were found. By contrast, *Fucus vesiculosus* was reported to have an increased content of glycolipids and reduced triacylglycerol in winter [3]. However, these changes could have been related to the season and developmental stage rather than ambient temperature and, moreover, we have found little change in the lipid pattern of *F. vesiculosus* harvested at different times of the year (A. L. Jones and J. L. Harwood, unpublished observations).

Although the alteration in fatty acyl unsaturation which frequently follows changes in growth temperature is the best known adaptive phenomenon, other alterations are possible. These include retailoring of molecular species, chain shortening and the alteration of membrane lipid quantity or quality [see 8]. Some of these changes have been reported in algae such as *Dunaliella salina* [8, 9] or cyanobacteria [10]. These changes often occur on different time-scales and some adaptations may be observed after very short periods.

As part of a study on the lipid biochemistry of two intertidal marine red algae, *Chondrus crispus* and *Polysiphonia lanosa*, the changes in lipid and fatty acid composition in response to seasonal fluctuations in water temperature were examined. Further complementary studies followed changes in the lipid metabolism *in vitro* at different temperatures by labelling lipids with [^{14}C]-acetate and monitoring the subsequent distribution of radioactivity.

RESULTS AND DISCUSSION

The acyl lipid composition of *P. lanosa* collected in January and August when the average water temperatures were 5 and 18°C, respectively (this is the normal annual range along the South Wales coast) is shown in Table 1. Increased amounts of all the major lipids were found in the summer months with the elevation in PC (+phosphatidylsulphocholine) being particularly marked. In contrast, minor lipids such as PG showed little change or, in the cases of non-esterified fatty acids and TAG, a significant decrease. The relative percentages of MGDG and PC were increased in the summer months while decreases were found for PG and the neutral lipids. The rather high concentrations of non-esterified fatty acids found in winter-collected algae may be a typical finding as it has been noted before that the amount of this

Abbreviations: MGDG, monoglycosyldiacylglycerol (mainly monogalactosyldiacylglycerol [12]); DGDG, diglycosyldiacylglycerol (mainly digalactosyldiacylglycerol [12]); SQDG, sulphoquinovosyldiacylglycerol; PC, phosphatidylcholine (this band also includes some phosphatidylsulphocholine (PSC) when separated by TLC [12]); PE, phosphatidylethanolamine; PG, phosphatidylglycerol; TAG, triacylglycerol.

Table 1. Comparison of *Polysiphonia lanosa* acyl lipid content in winter and summer

Acyl lipid	January (5°) μmol FA/ g dry wt	January (5°) % Total fatty acid	August (18°) μmol FA/ g dry wt	August (18°) % Total fatty acid
MGDG	8.4	17.6 ± 1.4	22.0	23.7 ± 1.1
DGDG	11.6	24.5 ± 5.0	19.4	20.9 ± 2.5
SQDG	5.6	11.9 ± 4.0	13.0	14.0 ± 1.5
PC + PSC	8.6	18.3 ± 4.4	28.1	30.3 ± 5.0
PG	2.4	5.0 ± 0.3	2.0	2.2 ± 0.2
PE	0.6	1.4 ± 0.2	2.0	2.1 ± 0.2
TAG	3.2	6.8 ± 1.9	0.6	0.6 ± 0.1
FFA	3.9	8.2 ± 1.7	0.7	0.8 ± 0.2
Others	3.1	6.6 ± 0.7	5.1	5.4 ± 0.7
Mass	13436 ± 6566		26366 ± 4440	

Values are means ± s.d. (n = 3–7). Sea temperatures for January and August are indicated in parenthesis. Mass = μg fatty acid/g dry wt, μmol FA/g dry wt = μmol fatty acid/g dry wt.

Lipid abbreviations: FFA = free (non-esterified) fatty acid. Other lipids include diphosphatidylglycerol, phosphatidic acid, sterol esters, monoacyl derivatives of glycerolipids and one unidentified lipid. MGDG and DGDG both resolved into double bands on TLC [12] but the two species have been combined in this Table.

class of lipid varies considerably with the season and nutrient availability [3]. The changes in PC, on the other hand, contrast with results for some higher plant tissues where this phosphoglyceride is increased by frost-hardening [1, 11]. However, this increase in PC is not a ubiquitous finding for plants [1].

The total fatty acid composition of *C. crispus* and *P. lanosa* at different times of the year is shown in Table 2. The amounts of C_{20} polyunsaturated fatty acids declined during the summer in *P. lanosa* whilst those of palmitate increased. This led to a reduced unsaturation index which is in accordance with results for higher terrestrial plants when temperatures rise [1]. In *C. crispus* the differences in composition between winter and summer appear to be smaller even though the sea temperatures were 4–5° and 14–18°, respectively. This may have been due to habitat as the alga tends to grow in rock pools close to freshwater outlets. Alternatively, it may suggest that factors other than temperature were having an influence, radiolabelling data from *in vitro* experiments indicated that rising temperature does increase saturation in this species (see Table 3).

As *P. lanosa* showed some obvious modifications in acyl lipid composition which were consistent with a response to environmental temperature, this alga was examined in more detail. A comparison of the fatty acyl content of the major endogenous lipids for material collected in January and August is shown in Table 4. For MGDG, double-banding was seen on TLC due to the partial separation of molecular species [12]; both fractions are shown in Table 4. Individual lipids were found to respond differently to endogenous temperature. Thus, whereas the slower (more saturated) MGDG showed only a slight increase in oleate and linoleate proportions in summer, the faster moving (more unsaturated) fraction showed increases in palmitate and decreases in arachidonate and eicosapentaenoate contents. By contrast, DGDG showed no significant changes, while SQDG had a much increased proportion of palmitate in summer. The two major phospholipids reacted differently. PC (+ phosphatidylsulphocholine) showed a large increase in palmit-

Table 2. Total fatty acid composition of *Chondrus crispus* and *Polysiphonia lanosa* in different seasons

Fatty acid	*Polysiphonia lanosa* Winter (4–5°)	*Polysiphonia lanosa* Summer (14–18°)	*Chondrus crispus* Winter (4–5°)	*Chondrus crispus* Summer (14–18°)
14:0	*1.8 ± 0.1	0.9 ± 0.1	*2.4 ± 0.4	0.7 ± 0.3
16:0	*19.4 ± 2.3	32.2 ± 0.4	22.2 ± 2.3	30.3 ± 3.5
16:1	6.6 ± 0.6	5.7 ± 0.1	4.2 ± 0.5	3.1 ± 0.4
18:0	1.1 ± 0.1	1.0 ± tr	2.2 ± 0.4	1.3 ± 0.4
18:1	13.3 ± 0.5	14.4 ± 0.2	9.4 ± 0.4	9.6 ± 0.8
18:2	4.7 ± 0.3	5.3 ± 0.1	1.8 ± 0.2	1.2 ± 0.3
18:3	0.5 ± tr	0.8 ± 0.1	1.2 ± 0.3	0.8 ± 0.2
18:4	0.4 ± tr	0.3 ± tr	1.1 ± 0.3	2.4 ± 1.1
20:4	10.2 ± 1.1	8.3 ± 0.1	25.0 ± 1.7	26.0 ± 1.8
20:5	*38.6 ± 2.6	28.0 ± 0.3	26.9 ± 1.7	23.1 ± 2.6
Others	3.4 ± 0.2	3.1 ± 0.1	3.6 ± 0.4	1.5 ± 0.7
Mass	*18429 ± 613	23766 ± 451	18875 ± 2830	22777 ± 4413
S.I.	2.7	2.1	2.7	2.4
Samples	5	21	5	6

Values are means ± s.e.m. Sea temperatures are indicated in parenthesis. Mass = μg fatty acid/g dry wt, S.I. = saturation index (average number of double bonds per acyl chain), tr = trace (< 0.05%). Statistical analysis was carried out using Student's t test; * = $p < 0.05$.

Fatty acid abbreviations: 14:0 = myristate, 16:0 = palmitate, 16:1 = palmitoleate, 18:0 = stearate, 18:1 = oleate, 18:2 = linolcate, 18:3 = α-linolenate, 18:4 = octadecatetraenoate (n-3), 20:4 = arachidonate, 20:5 = eicosapentaenoate (n-3).

Table 3. Effect of temperature on incorporation of radioactivity from [^{14}C]-acetate into fatty acids of *Chondrus crispus* and *Polysiphonia lanosa*

	Incub. time (hr)	Temp. (°)	Distribution of radioactivity (% ^{14}C-fatty acids)						% Total saturates
			14:0	16:0	16:1	18:0	18:1	Others	
C. crispus	1	4	tr.	14	11	tr.	76	tr.	41
		15	12	33	5	22	27	1	68
	4	4	4	10	13	7	66	tr.	21
		15	6	23*	5*	13	51	2	43*
	24	4	1	28	6	12	53	1	41
		15	10*	37	5	11	34*	3	59*
P. lanosa	24	4	3	23	15	3	36	11	30
		15	8*	36	11	10*	29	6	55*

Values are means ($n = 3$) except for 1 hr at 15° where $n = 1$. For simplicity, standard deviations are omitted but statistical analysis was by Student's t test for the 4 and 24 hr samples, * = P 0.05.

[^{14}C]-acetate was supplied in a 1 hr pulse for *C. crispus*. For *P. lanosa* [^{14}C]-acetate was present throughout the 24 hr incubations. All samples were illuminated. tr = trace (< 0.5%). See Table 2 for fatty acid abbreviations.

ate and lowered amounts of oleate and eicosapentaenoate. In contrast, PG showed reciprocal changes in palmitate and hexadecenoate (*trans*, *n*-13) and, surprisingly, a significant increase in eicosapentaenoate contents—due in part to the disappearance of minor components in the summer. The neutral lipid fractions, TAG and unesterified fatty acids, showed little seasonal variation in fatty acid content.

The general trend shown in the above results was, therefore, that most membrane lipids showed an increased saturation at higher growth temperatures as would be expected. However, the nature of the changes were different from lipid to lipid indicating subtle control mechanisms. Because the physical nature of neutral acyl lipids is of less importance for their function, it is not surprising that they show less adaptation to growth temperature than membrane lipid constituents.

To examine some of the effects of temperature on lipid metabolism under more controlled conditions and to confirm the results from field-collected material, pulse-chase experiments using [^{14}C]-acetate were carried out with *C. crispus* at 4, 15 and 25°. Wide variations in [^{14}C]-acetate uptake in *C. crispus*, particularly at lower temperatures, appear typical for this alga, whereas other marine algae show less variation (unpublished observations). This could, in part, be due to *C. crispus* having a rather high sensitivity to environmental stress, particularly dessication. Its normal habitat is in rock-pools and running streams on the beach which prevent it drying out as the tide retreats. Most other intertidal algae we have studied appear able to tolerate this drying out with minimal effects on metabolic rates after re-immersion. Weighing and general handling of *C. crispus* cause a certain amount of dessication and this could have been sufficient to introduce a variation in the metabolic rate of individual samples of this alga until rehydration had taken place fully.

Raising the incubation temperature from 4 to 15° (the optimum temperature for this alga as determined by photosynthetic measurements; unpublished data) increased total [^{14}C]-acetate incorporation by *ca* three-fold while another rise to 25° caused a further three-fold increase (Table 5). About half of the total lipid counts were found in acyl lipids—pigments accounting for the bulk of the remainder. At 4° the label in these acyl lipids was almost entirely located in their acyl moieties, but with increasing incubation temperature more label became incorporated into non-acyl components. This was probably due to increased metabolic rate and hence more rapid labelling of the glycerol and monosaccharide pools. This increased metabolism might explain why high proportions of radiolabel were incorporated into lipids at 4 and 15°, whereas at 25° only 30% of the total label taken up was in lipids at 24 hr in comparison to 70–84% at the lower temperatures.

The percentages of radiolabel in the major acyl lipids after incubations at 4 and 15° are shown in Table 6. Incubations at 25° appeared to adversely affect metabolism in this alga, leading to a highly variable distribution of label between the lipids (data not shown). This might be expected, as 25° is clearly outside the normal temperature range experienced by *C. crispus*.

Both DGDG and SQDG increased their relative rates of [^{14}C]-incorporation at 15° compared to 4°. In contrast, PG was relatively less labelled at all time points with algae incubated at 15° compared to 4°. No obvious changes in the proportions of radiolabel in PC (+phosphatidylsulphocholine) and PE were seen in *C. crispus*. This result, together with the seasonal data for *P. lanosa* (Table 1), tends to suggest that mechanisms similar to those involved in frost hardening, where PC and PE are synthesized at increased rates [11, 13], are probably not of importance in these algae.

The increased labelling of SQDG and DGDG at higher temperatures may be due to turn-over of their acyl chains in connection with mechanisms for the constant regulation of membrane fluidity. Indeed, modification of the acyl chain composition of SQDG in wheat thylakoids has been observed during temperature adaptation [14]. The increased proportions of SQDG and DGDG (both typical chloroplast lipids) labelled at 15° may also indicate a role for them in the regulation of chloroplast membrane fluidity or in photosynthetic electron transport and/or light harvesting, possibly by stabilizing certain components involved in these processes [see 15, 16]. The optimum temperature for photosynthesis in *C. crispus* is

Table 4. Comparison of fatty acid composition of major acyl lipids for *Polysiphonia lanosa* harvested in summer and winter

Acyl lipid	Fatty acid composition (% total acids) 14:0	16:0	†16:1 (*n*-7)	18:0	18:1 (*n*-9)	18:2 (*n*-6)	18:3 (*n*-6)	18:3 (*n*-3)	18:4 (*n*-3)	20:4 (*n*-6)	20:5 (*n*-3)	Others	S.I.
(W)	0.2	27.4	3.4	*0.8	*4.3	*3.9	*0.5	1.0	*1.9	4.9	46.5	*5.2	2.9
MGDG1	±0.2	±5.0	±0.9	±0.7	±0.7	±0.2	±0.2	±0.3	±0.5	±1.4	±5.1	±1.7	
(S)	0.3	29.1	1.9	2.2	8.1	7.4	nd	0.5	nd	6.3	43.5	0.7	2.7
	±0.2	±7.3	±0.5	±0.5	±1.5	±1.2		±0.2		±1.2	±9.0	±0.3	
(W)	tr	**2.0	**0.3	**0.3	**1.0	**0.9	0.1	**tr	*0.2	*13.4	**80.3	1.5	4.6
MGDG2		±0.5	±tr	±0.1	±0.2	±0.2	±tr		±0.1	±1.2	±1.9	±0.4	
(S)	0.1	15.9	1.9	1.6	3.2	4.4	0.1	t2	0.6	9.4	60.5	1.1	3.6
	±tr	±0.5	±0.2	±0.1	±0.3	±0.3	±tr	±0.2	±0.1	±0.7	±1.0	±0.3	
(W)	0.8	28.5	6.6	0.9	8.3	4.5	*0.6	0.3	*0.8	5.4	40.5	*2.8	2.6
DGDG	±0.6	±10.0	±2.4	±0.5	±4.5	±2.0	±0.3	±0.1	±0.3	±3.4	±9.9	±0.6	
(S)	0.8	32.6	4.7	0.7	7.2	7.4	nd	0.5	0.1	4.1	40.9	1.0	2.6
	±0.5	±2.9	±0.6	±0.2	±0.9	±1.0		±0.1	±tr	±0.3	±3.8	±0.3	
(W)	3.5	28.5	*10.8	0.7	15.2	3.4	*0.5	0.3	0.5	6.3	27.2	*3.1	2.0
SQDG	±2.0	±13.0	±1.9	±0.3	±2.4	±0.9	±0.2	±0.1	±0.3	±1.4	±7.7	±0.8	
(S)	2.9	44.7	5.8	0.5	13.1	3.6	0.1	0.4	0.3	4.5	23.7	0.4	1.7
	±2.0	±2.6	±0.2	±0.2	±1.6	±0.6	±tr	±0.1	±0.1	±0.3	±1.4	±0.1	
(W)	*0.2	**4.5	*1.5	1.2	*19.0	*2.7	*2.5	tr	0.8	16.8	*45.2	*5.6	3.4
PC+PSC	±0.3	±0.9	±0.3	±1.0	±1.0	±0.3	±0.5		±0.4	±1.9	±2.6	±1.7	
(S)	2.1	20.5	3.8	1.2	16.1	5.2	0.9	0.1	0.2	15.3	32.8	1.8	2.6
	±0.5	±1.7	±0.2	±1.0	±1.3	±0.9	±0.5	±tr	±tr	±0.5	±1.4	±0.5	
(W)	1.7	*15.4	*12.1	6.8	17.1	*0.8	*2.9	1.8	*7.4	2.4	**19.0	*12.6	2.0
PG	±1.5	±3.6	±2.1	±4.8	±5.8	±0.4	±1.6	±0.9	±1.0	±1.4	±3.8	±5.1	
(S)	nd	24.4	8.5	3.9	18.2	2.2	0.2	1.4	1.6	4.0	35.0	0.6	2.4
		±2.9	±0.2	±1.9	±3.3	±0.2	±0.1	±0.2	±0.3	±0.6	±1.9	±0.2	
(W)	0.5	11.5	**8.9	2.8	14.0	5.7	0.1	1.4	tr	12.0	34.6	8.5	2.9
TG	±0.4	±2.4	±3.1	±0.3	±2.4	±1.2	±tr	±0.5		±1.6	±9.3	±2.2	
(S)	0.1	12.8	3.3	3.3	11.9	6.2	nd	1.0	nd	15.9	36.7	8.8	2.9
	±0.1	±3.1	±1.4	±1.6	±0.2	±1.4		±0.3		±1.7	±3.3	±2.7	
(W)	3.3	30.1	4.2	13.1	31.3	2.9	*0.6	*0.7	**4.7	0.7	1.8	6.6	0.9
FFA	±0.9	±5.5	±2.1	±0.8	±6.7	±0.5	±0.3	±0.3	±2.4	±0.7	±1.8	±2.2	
(S)	5.5	27.6	2.5	10.5	42.5	1.4	tr	tr	nd	0.2	0.2	9.6	0.6
	±1.7	±2.9	±0.7	±4.3	±4.2	±1.3				±tr	±0.1	±2.0	

Values are means ±s.d. ($n=3$) for material collected in January to March (W) and in August (S). tr = trace (<0.05%), nd = not detected.

† *n*-7 isomer except for PG where it is the 16:1 *trans n*-13 isomer.

Abbreviations as Tables 1 and 2 except that the two MGDG bands are shown with MGDG1 being the slower migrating fraction. Statistical analysis was carried out using Student's *t* test.

* = $P < 0.05$.

** = $P < 0.01$.

15° so at this temperature it could be expected that the photosynthetic components would show high rates of turn-over and metabolism compared to 4°. This might require a commensurate change in the metabolism of those chloroplast lipids involved in photosynthetic membrane structure and function. Changed DGDG:MGDG ratios have been observed in some higher plants such as frost resistant wheat [14] and pine [17] during temperature adaptation. However, the ratio was increased rather than decreased as in *C. crispus*. Moreover, other plants such as pea, showed no change [18]. Thus, as yet, there is no general agreement on the changes in lipid composition which different plants make in response to varying environmental temperatures.

The increased proportions of labelled, saturated fatty acids (myristate, palmitate, stearate) and relatively decreased labelling of oleate in *C. crispus* as temperatures rose from 4° to 15° (Table 3) agreed well with results from other marine algae [3, 5] and higher plants [1]. *Polysiphonia lanosa* showed a similar response to temperature. These changes have been linked to the maintenance of membrane fluidity [19] by altering the activity or amounts of the desaturating enzymes. However, there is also some evidence that it merely reflects decreased oxygen solubility at higher temperatures, making aerobic desaturation less efficient and, hence, resulting in greater proportions of saturated fatty acids [20]. The reaction of *C. crispus* to different incubation temperatures is rapid and is clearly visible after only 1 hr. This result may suggest that substrate (oxygen) availability is an important control in regulating desaturation.

The pulse-chase data for *C. crispus* (Table 3) showed that the pattern of labelled fatty acids varied during the incubation period. Interestingly, the longest incubations at 4° resulted in a higher proportion of radiolabel in saturated fatty acids. This may have been due to a higher

Table 5. The effects of temperature on the uptake and distribution of radiolabel from [^{14}C]-acetate in *Chondrus crispus*

Temp. (°)	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		42 ± 19	58 ± 32	38 ± 10	100 ± 17
4	4	1.3 ± 0.3	32 ± 5	68 ± 9	43 ± 5	100 ± 27
	24		30 ± 12	70 ± 25	34 ± 4	100 ± 25
	1		52	48	38	96
15	4	3.8 ± 0.7	30 ± 5	70 ± 26	45 ± 5	96 ± 6
	24		16 ± 6	84 ± 40	51 ± 4	86 ± 17
	1		83 ± 28	17 ± 8	nd	nd
25	4	13.9 ± 1.5	49 ± 27	51 ± 12	nd	nd
	24		70 ± 26	30 ± 7	nd	nd

Values are means ±s.d. (n = 3–15) except for 1 hr at 15° where n = 1. All samples were illuminated. [^{14}C]-acetate was supplied in a 1 hr pulse. Total uptake (dpm/g fr. wt × 10^{-6}) determined from the initial activity of the incubation soln minus the final activity of the soln. nd = not determined.

Table 6. Incorporation of radiolabel from [^{14}C]-acetate into the acyl lipids of *Chondrus crispus* at 4 and 15°

	% Distribution of radioactivity (% ^{14}C-lipids) Incubation time (hr)					
	1		4		24	
	4°	15°	4°	15°	4°	15°
MGDG1	1.3	7.5	2.5	6.8	9.1	5.5
MGDG2	0.8	2.2	1.7	2.7	6.4	5.1
DGDG	1.6	12.0	*2.7	8.4	*4.0	8.7
SQDG1	0.3	7.9	*1.1	8.7	*1.8	8.0
SQDG2	0.5	3.7	*0.8	4.2	*1.6	4.3
PG	56.4	41.2	*49.9	35.4	*32.3	23.3
PC(+PSC)	17.9	11.8	*18.9	14.0	16.5	14.6
PE	3.7	2.0	2.3	3.4	3.2	5.2
DPG/PA	3.6	2.8	5.4	3.4	*1.7	5.7
Others	13.9	8.9	14.7	12.8	23.4	19.4

Values are means (n = 3) except for 1 hr at 15° where n = 1. For simplicity, standard deviations are omitted but statistical analysis was by Student's t test for the 4 and 24 hr samples, * = P 0.05. [^{14}C]-acetate supplied in a 1 hr pulse. All samples were illuminated. DPG = diphosphatidylglycerol, PA = phosphatidic acid. For other lipid abbreviations see Table 1. MGDG and SQDG both separated into two bands by TLC with the slower moving band designated 1.

turnover of oleate compared to the saturated fatty acids. Again, it suggests that increased unsaturation at lower temperatures does not depend on the induction of more desaturase enzyme protein, since there was no evidence from the data (Table 3) for increased desaturation with time. Polyunsaturated fatty acids were only poorly labelled even after 24 hr. Such a result has also been reported for marine brown algae [21].

It is clear from the above results that marine red algae, like most other poikilotherms, react to changes in environmental temperature by adjusting their lipid content and metabolism. We have attempted to find rationales for these changes either in terms of membrane fluidity or in preservation of general membrane (particularly thylakoid) functions. However, more work is clearly needed in order to provide further evidence that the changes noted are true adaptations and not just responses to temperature alterations [see 2]. Moreover, the experiments should be expanded to evaluate lipid molecular species in order to see whether retailoring constitutes an important short-term adaptive mechanism [8]. The red algae with their primitive photosynthetic membrane structure and highly variable habitats offer good experimental systems in which to probe such adaptive phenomena in more detail.

EXPERIMENTAL

Fatty acid standards were obtained from Nu-Check Prep. Inc. (Elysian, USA) and lipid standards from Sigma or isolated from leaf tissue [16]. [1-^{14}C]-Acetate was obtained from Amersham.

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 photoperiod 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 noticeable change in morphology or metabolism.

Isotope labelling. Tissue samples of *ca* 50 mg fr. wt were washed in 0.5% Triton X-100 to remove surface microbial contamination [22], blotted dry and weighed before placing in a screw-capped test tube containing 3 ml sterile seawater. [1-^{14}C]-Acetate was added (usually 1 μCi, 11.7 mM) and the sealed tube placed in a shaking H_2O bath at 4, 15 or 25°. Illumination was provided (*ca* 200 μE/m^2/sec) using warm white fluorescent tubes. After 1 hr the tissue was removed from the medium, rinsed and returned to fr. sterilized seawater (with unlabelled acetate; 11.7 mM) for the remaining incubation time. In a few cases time-course expts (where radiolabel was not removed after 1 hr) were performed instead of pulse-chase expts. An aliquot of the radiolabelled incubation medium was taken for scintillation counting to determine the total uptake of radiolabel. After incubation the

lipids were extd [12] and divided into two equal frs. One fr. was transmethylated and analysed by radio-GC, the other was sepd into individual lipids by CC and TLC [12].

Routine lipid identification was by reference to authentic standards. Individual lipids were identified by the use of specific spray reagents [23, 24], acid and alkaline degradation [23] and autoradiography after labelling with ^{32}P and ^{35}S [12].

Fatty acid Me esters, prepd by transmethylation with 2.5% (v/v) H_2SO_4 in dry MeOH at 70° were sepd as previously described [12]. Identification was made routinely by ref. to authentic stds but more complete identification had been made previously [12].

Radiolabelled samples were counted in a scintillant of PCS (Amersham)–xylene (2:1). Quench corrections were made using the ext. std method.

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