Phytochemistry, Vol. 28, No. 11, pp. 2943–2948, 1989.
Printed in Great Britain.

0031-9422/89 $3.00 + 0.00

EFFECT OF TEMPERATURE ON LIPID COMPOSITION OF THE MARINE CRYPTOMONAD *CHROOMONAS SALINA*

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(*Received* 13 *February* 1989)

Key Word Index—*Chroomonas salina*; cryptomonad; lipid composition; temperature.

Abstract—The lipid compositions of the marine cryptomonad *Chroomonas salina* cultured at 20 and 8° were compared. Algae cultured at 8° had a higher lipid content than those maintained at 20°. Triacylglycerols (TAG) were the major lipid class at both temperatures. The proportion of sterols in total lipid exhibited the most significant change in relation to temperature. Saturated fatty acids always predominated in TAG although their proportion was decreased, and that of monoenes increased, by culturing the alga at 8°. Monogalactosyldiacylglycerol and digalactosyldiacylglycerol, particularly the latter, were characterised by high levels of C_{18} polyunsaturated fatty acids (PUFA). Culturing algae at 8° resulted in significant increases in the proportion of 18:4(*n*-3) with corresponding decreases in those of 18:2(*n*-6) and 18:3(*n*-6), in both these galactolipids. PUFA also predominated in phospholipids but the levels of 20:5(*n*-3) and 22:6(*n*-3) were higher than in galactolipids. The magnitude of changes in fatty acid composition in relation to temperature was smaller in phospholipids than in galactolipids.

INTRODUCTION

It is well established that an inverse relationship exists in higher plants between growth temperature and the degree of unsaturation of fatty acids in component lipids [1, 2]. Increases in unsaturation in response to low environmental temperatures are usually associated with the polar lipids of biomembranes although the proportions of polyunsaturated fatty acids in the triacylglycerols of *Hibiscus* leaves also show minor changes in response to seasonal temperature changes [3].

In photosynthetic microalgae the occurrence of changes in fatty acid composition in response to environmental temperatures has only been established for a few species [4–7]. Such studies with microalgae have considered only total lipid and have not examined changes within the different lipid classes. We have found previously that the level of polyunsaturated fatty acids is higher in total polar lipids from cultures of *Chroomonas salina* grown and aged at 8° than at 20° [8]. The polar lipids of photosynthetic microalgae such as *C. salina* includes both phospholipids and glycolipids, the latter class of lipids being associated with photosynthetic membranes [9, 10]. Neutral lipids, particularly triacylglycerols, also feature in algal lipids. The extent to which the fatty acid compositions of the different lipid classes is influenced by temperature is unknown.

The present study was undertaken to determine the detailed lipid composition of *C. salina* and to establish the manner in which the fatty acid composition of different lipid classes changes in response to a lowering of environmental temperature.

RESULTS

Cultures of *C. salina* maintained at 20 and 8° had lag phases of two and five days respectively. The growth rate of the algae was greater at 20° than 8° (Table 1) and the cell density reached by the onset of stationary phase was notably higher at 20° than 8°. When harvested the three cultures maintained at 20° were all identical in colour whereas one of the cultures at 8° was noticeably paler than the two others grown at this temperature.

Chroomonas salina cultured at 8° contained a significantly higher proportion of its dry weight as lipid than that cultured at 20° (Table 1). Regardless of growth temperature, triacylglycerols (TAG) were the principal

Table 1. Effect of temperature on growth rate and lipid content of *C. salina*

Temp°	Initial cell no. ($\times 10^{-5}$)	Final cell no. ($\times 10^{-6}$)	Time* (days)	Lipid content (% dry weight)
20	0.86	4.83 ± 0.54	8	14.9 ± 0.7
8	0.86	1.99 ± 0.77	17	21.9 ± 2.1†

*Time to onset of stationary phase.
Values are means ± s.d. of three cultures.
†Significantly different from 20° value, $p \leq 0.01$.

lipid class present, accounting for over 40% of the total lipid (Table 2). Sterols were the second most abundant lipid in cultures from both temperatures. The galactolipid, digalactosyldiacylglycerol (DGDG) was the major polar lipid class, comprising some 8.5% of the total lipid. Monogalactosyldiacylglycerols (MGDG) accounted for 6.3 and 4.6% of lipid in *C. salina* cultured at 20 and 8° respectively. The levels of DGDG and MGDG in total lipid were not significantly affected by temperature.

Phosphatidylethanolamine (PE) was only a minor component at both temperatures. Under the HPTLC conditions employed, phosphatidylglycerol (PG) and cardiolipin (CL) could not be separated and together made up *ca* 2.5% of the total lipid from all cultures. Similarly, phosphatidylserine (PS) and phosphatidylinositol (PI) could not be completely resolved. Specific staining with Dragendorff's reagent showed phosphatidylcholine (PC) to be present as two distinct bands: one (PC_1) had R_f 0.13 and the other R_f 0.23 in the first solvent system of the HPTLC system used. PC_2 was coincident with PS and PI. The most significant change in relation to growth temperature was the decreased proportion of sterols observed at 8°. Free fatty acids also occurred at a significantly lower level in total lipid at 8°. The only significant increase in relation to temperature was that observed in the proportion of the band containing PS, PI and PC_2 which increased from 2.1% at 20° to 5.9% at 8°.

Growth temperature influenced the fatty acid composition of the lipid classes examined. In TAG, the major lipid class, 14:0 was the principal saturate and 18:1(*n*-9) the major monoene regardless of growth temperature (Table 3). The proportions of 16:0 and 18:0 were significantly lower, and those of 18:1(*n*-9) and 18:1(*n*-7) significantly higher, in TAG at 8° compared with that at 20°. Overall, the TAG were richer in monoenes and poorer in saturates at 8 than 20°. The major polyunsaturated fatty acid (PUFA) in TAG at 20°, was 18:2(*n*-6) accounting for 20.4% of total fatty acids, but comprised only 8.3% at 8°. The proportions of 18:3(*n*-6) and 20:4(*n*-6) were also lower at 8 than 20°. The corresponding increases in the proportions of 18:4(*n*-3) resulted in there being no overall change in the level of PUFA in TAG with temperature.

Table 2. Effect of temperature on lipid class composition of *C. salina*

	% total lipid	
	20°	8°
Steryl/wax esters*	4.9±0.9	4.3±2.3
Triacylglycerols	45.2±2.0	47.5±9.9
Free fatty acids	5.0±0.6	2.2±0.3‡
Sterols*	13.4±0.1	9.2±0.7†
Pigments	2.9±0.1	5.5±2.2
MGDG*	6.3±0.4	4.6±1.1
Unknowns	1.5±0.1	1.2±0.1§
DGDG	9.4±0.3	8.0±1.1
PE	0.5±0.1	0.5±0.1
PG+CL	2.5±0.3	3.0±1.1
Sulpholipids	2.2±0.1	2.1±0.6
PS+PI+PC_2	2.1±0.4	5.9±1.8§
PC_1	3.9±0.2	5.9±1.6

Values are means±s.d. of three cultures.

*Contained traces of pigments.

Significantly different from 20° value, †$p \leq 0.001$; ‡$p \leq 0.002$; §$p \leq 0.05$.

MGDG, monogalactosyldiacylglycerols; DGDG, digalactosyldiacylglycerols; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CL, cardiolipin; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine.

In MGDG from *C. salina* grown at 20°, 18:2(*n*-6) was the principal fatty acid, comprising 24.8% of the total. Growing the alga at 8° resulted in the proportion of 18:2(*n*-6) in this lipid class being halved. In contrast, those of 18:3(*n*-3) and 18:4(*n*-3) increased from 11.7% to 21.1% and 7.5% to 32.9% respectively. The overall content of PUFA in MGDG was significantly lower (56.1%) in the alga at 20° than at 8° (75.3%). The proportions of 14:0 and 16:0 in MGDG were both higher at 20° than 8° and thus the overall level of saturates decreased from 19.8% at 20° to 9.3% at 8°. The major monoene present in MGDG at both temperatures, was 18:1(*n*-7) accounting for 12.9% at 20°. The monoene content of MGDG was higher at 20° than 8°, due mainly to decreases in 18:1(*n*-9) and 18:1(*n*-7) with temperature.

In comparison with MGDG at 20°, DGDG at the same temperature contained less saturated and monoenoic fatty acids and was correspondingly richer in PUFA. In particular, the proportions of 18:2(*n*-6), 18:3(*n*-6), 18:3(*n*-3) and 18:4(*n*-3) in DGDG were all approximately double those in MGDG; 20:5(*n*-3) and 22:6(*n*-3) were present at levels of 2.2 and 5.0% respectively in MGDG but only 0.3 and 0.6% in DGDG. The proportions of individual PUFA in DGDG were influenced greatly by temperature. The largest difference was observed with 18:2(*n*-6) which accounted for 40.1% at 20° but only 11.3% at 8°. Conversely, the proportion of 18:4(*n*-3) in DGDG was 14.7% at 20° and 45.8% at 8°. The level of 18:3(*n*-3) was not significantly affected by temperature. The overall PUFA content was similar at over 85% for both temperatures. The level of saturates in DGDG was significantly higher at 20 than 8° whereas that of monoenes was very similar at both temperatures.

A notable difference between phospholipids and galactolipids in *C. salina* was that 20:5(*n*-3) and 22:6(*n*-3) were major PUFA in phospholipids whereas 18C PUFA predominated in the galactolipids. In PC_1 at 20°, 20:5(*n*-3) was the major fatty acid comprising 22.2% of the total and 22:6(*n*-3) was the next most abundant at 16.8% (Table 4). Growth temperature did not alter significantly the proportions of either of these PUFA. The proportions of 18:2(*n*-6), 18:3(*n*-6) and 18:3(*n*-3) were all significantly lower at 8 than 20°. The proportion of 18:4(*n*-3) was higher but not significantly, and overall, temperature did affect the total PUFA content of this lipid class. Likewise, the levels of saturates (19.4%) and monoenes (4.2%) in PC_1 were not influenced by temperature.

At 20°; 22:6(*n*-3) accounted for 24.5% and 20:5(*n*-3) 9.4% of the fatty acids of the PS+PI+PC_2 fraction. The most abundant C18 PUFA was 18:2(*n*-6), at a level of 11.5%. In comparison with PC_1 this fraction was richer in monoenes most notably 18:1(*n*-7) which made up 10% of the total fatty acids at 20°. Lowering the growth temperature resulted in significant decreases in the proportions of 16:0, 18:1(*n*-9), 18:1(*n*-7), 18:2(*n*-6), 18:3(*n*-6), 18:3(*n*-3), and 20:4(*n*-6) and increases in those of 18:4(*n*-3), 20:4(*n*-3), 20:5(*n*-3) and 22:6(*n*-3).

Table 3. Effect of temperature on the fatty acid composition (weight %) of triacylglycerols and galactolipids of *C. salina*

	TAG		MGDG		DGDG	
	20°	8°	20°	8°	20°	8°
12:0	0.8±0.6	0.5±0.3	—	—	—	—
14:0	21.6±1.1	22.5±1.1	10.9±0.9	4.3±0.5*	3.1±0.2	1.7±0.2*
15:0	0.3±0.0	0.4±0.1	0.3±0.1	0.2±0.1	0.1±0.0	0.1±0.0
16:0	18.5±0.7	10.8±0.8*	7.4±0.6	4.0±05†	5.1±0.4	2.6±0.3*
16:1(*n*-7)	2.2±0.1	3.4±0.2*	2.6±0.8	4.9±3.1	0.8±0.1	0.5±0.1‖
16:1(*n*-13) *trans*	—	—	—	—	—	—
16:2	0.7±0.1	0.1±0.0*	—	—	—	—
16:3	—	—	—	—	—	0.2±0.1‖
16:4	—	—	—	0.9±0.9	—	0.3±0.1‡
18:0	1.2±0.1	0.5±0.0*	1.2±0.3	0.8±0.1	0.4±0.1	0.2±0.1
18:1(*n*-9)	15.3±0.9	21.6±1.0*	7.7±0.8	2.7±0.1*	3.7±0.4	2.2±0.5§
18:1(*n*-7)	3.2±0.3	4.4±0.3‡	12.9±1.7	7.9±3.1	1.0±0.1	1.4±0.6
18:2(*n*-6)	20.4±0.6	8.3±0.4*	24.8±1.5	12.4±2.9‖	40.1±3.8	11.3±2.9*
18:3(*n*-6)	1.2±0.2	0.7±0.2‖	4.1±0.3	2.9±0.7	8.5±0.4	4.3±1.4‡
18:3(*n*-3)	8.0±0.6	14.0±0.9*	11.7±0.8	21.1±5.4‖	21.4±0.9	26.5±3.3
18:4(*n*-3)	2.9±0.7	9.2±0.9*	7.5±1.7	32.9±5.9†	14.7±2.9	45.8±1.9*
20:1(*n*-9)	0.1±0.0	—	0.2±0.1	—	—	1.8±0.3*
20:3	—	0.1±0.0	—	—	—	—
20:4(*n*-6)	0.7±0.1	0.2±0.0*	—	0.1±0.0	—	—
20:4(*n*-3)	0.1±0.1	0.4±0.1‖	0.2±0.1	0.3±0.1	—	0.1±0.0
20:5(*n*-3)	1.4±0.4	2.0±0.3	2.2±0.4	1.5±0.6	0.3±0.0	0.6±0.2
22:5(*n*-3)	0.1±0.0	0.1±0.1	0.2±0.1	—	—	—
22:6(*n*-3)	0.7±0.1	0.5±0.2	5.0±0.6	3.2±1.6	0.6±0.3	0.5±0.2
Total sats	42.6±1.6	35.0±1.7‡	19.8±0.8	9.3±0.9*	8.7±0.2	4.6±0.4*
Total monos	20.8±0.6	29.4±0.9*	24.0±1.0	15.5±4.4‖	5.5±0.5	5.9±0.8
Total PUFA	36.2±1.5	35.6±1.2	56.1±0.7	75.3±5.6‡	85.6±0.3	89.6±1.3

Values are means±s.d. of three cultures.

Significantly different from 20° value, *$p \leq 0.001$; †$p \leq 0.002$; ‡$p \leq 0.01$; §$p \leq 0.02$; ‖$p \leq 0.05$.

TAG, triacylglycerols; MGDG, monogalactosyldiacylglycerols; DGDG, digalactosyldiacylglycerols; sats, saturates; monos, monoenes; PUFA, polyunsaturated fatty acids.

Of the fatty acids present in PE of algae grown at 20°, 69.9% were 22:6(*n*-3). No other PUFA exceeded 5%, while 11.5% of the total fatty acids were saturates of which 14:0 and 16:0 were the major components. The monoenes 16:1(*n*-7), 18:1(*n*-9) and 18:1(*n*-7) together comprised only 3.2% of the PE fatty acids. The PE derived from the pale coloured culture at 8° was notably different in fatty acid composition from the other two grown at this temperature. Consequently mean values for 8° samples had large s.d. and the significance of observed differences between 20 and 8° were reduced.

The fatty acid 16:1(*n*-13) *trans* was found in only PG + CL + SL and at a level of less than 5%, 16:0 and 18:2(*n*-6) accounted for 23.7% and 21.1% respectively of the fatty acids of this fraction at 20°. At this temperature the proportions of 18:3(*n*-3) (16.0%) and 18:4(*n*-3) (14.4%) exceeded that of 20:5(*n*-3) (10.8%), and 22:6(*n*-3) accounted for only 1.9% of the total fatty acids. The most significant difference between the PG + CL + SL fraction of 20 and 8° algae was the much lower level of 18:2(*n*-6) (4.8%) present at 8°. The levels of 18:4(*n*-3) and 20:5(*n*-3) were correspondingly higher at 8°. Of the four phospholipid fractions examined, only PS + PI + PC_2 exhibited changes overall in content of saturates, monoenes and PUFA in relation to growth temperature.

DISCUSSION

In the present study the lipid composition of the *C. salina* was determined at only one point in the growth cycle, namely late log/early stationary phase. Although the present results demonstrate that TAG are the predominant lipid class in *C. salina* at this stage, they do not provide information on changes in lipid composition throughout the growth cycle. The pattern of lipids present in photosynthetic microalgae has been shown to change during the growth phase and it is generally considered that the formation of TAG commences towards the end of the growth period [11]. The absence of large amounts of the wax esters which are known to accumulate in aging cultures of *C. salina* is in keeping with these lipids only being formed during late stationary phase [8, 12]. The level of saturates observed in TAG at 20° in this study is less than that observed previously for cultures of *C. salina* maintained at the same temperature for five weeks [8] and conforms to the general principal that TAG become more saturated with increasing culture age [11].

The polar lipid classes present in *C. salina* are typical of those reported for other microalgae [9, 10]. However the lack of quantition of these lipid classes in previous reports does not permit a comparison of the relative amounts in *C. salina* with other species.

Table 4. Effect of temperature on the fatty acid composition (weight %) of phospholipid classes of *C. salina*

	PC_1		$PS/PI/PC_2$		PE		PG/CL/SL	
	20°	8°	20°	8°	20°	8°	20°	8°
12:0	—	—	—	—	—	—	—	—
14:0	9.4±0.7	9.0±1.7	13.5±0.8	13.8±0.7	5.8±4.2	1.8±0.5	2.0±0.2	4.1±2.2
15:0	0.2±0.1	0.3±0.1	0.5±0.1	0.4±0.1	0.2±0.1	0.5±0.1‖	0.2±0.0	0.3±0.0
16:0	9.6±0.2	8.3±3.2	9.3±0.9	6.0±0.3‡	4.9±0.8	5.5±3.4	23.7±1.0	20.6±1.9
16:1(*n*-7)	1.4±0.1	1.1±0.3	1.6±0.2	1.3±0.5	1.6±0.5	2.2±0.5	0.3±0.1	0.6±0.2
16:1(*n*-13) *trans*	—	—	—	—	—	—	3.4±1.3	4.6±1.1
16:2	—	—	—	—	—	—	—	—
16:3	—	—	0.1±0.0	—	0.1±0.0	0.3±0.2	—	—
16:4	—	—	—	—	—	—	0.1±0.1	0.2±0.0
18:0	0.2±0.0	1.8±2.8	0.2±0.1	0.8±1.1	0.6±0.0	5.6±3.1‖	0.1±0.1	0.4±0.3
18:1(*n*-9)	1.8±0.2	1.1±1.1	1.1±0.1	0.3±0.1†	0.9±0.3	3.5±1.5‖	2.2±0.1	0.8±0.3†
18:1(*n*-7)	1.0±0.2	1.3±0.7	10.0±0.3	8.5±0.3‡	0.7±0.3	1.6±0.9	1.6±0.2	2.0±0.3
18:2(*n*-6)	9.3±1.1	1.8±1.5†	11.5±0.7	1.7±0.3*	5.0±0.2	1.8±0.6†	21.1±1.6	4.8±0.2*
18:3(*n*-6)	1.8±0.1	0.2±0.1*	1.1±0.2	0.2±0.1†	0.8±0.1	0.5±0.1‖	1.4±0.3	0.3±0.1‡
18:3(*n*-3)	6.4±0.8	3.5±0.7‡	7.9±0.8	5.1±0.2‡	3.4±0.7	3.8±1.3	16.0±0.9	18.1±1.7
18:4(*n*-3)	9.8±1.4	15.8±5.4	3.8±0.7	6.2±0.9‖	3.6±2.0	6.2±2.8	14.4±1.1	19.6±1.1‡
20:1(*n*-9)	—	0.9±0.6	—	—	—	1.0±0.8	—	0.6±0.3
20:3	0.6±0.1	0.9±0.0‡	0.3±0.1	0.3±0.1	0.1±0.0	—	—	—
20:4(*n*-6)	6.7±1.0	2.7±2.3	3.3±0.5	1.2±0.6‡	0.4±0.1	2.3±3.5	0.7±0.1	0.3±0.1‡
20:4(*n*-3)	1.5±0.2	7.6±0.5*	0.8±0.1	4.4±1.2‡	0.1±0.0	0.4±0.2†	0.1±0.0	0.8±0.5‡
20:5(*n*-3)	22.2±0.4	27.4±5.6	9.4±0.5	16.2±1.3†	2.0±0.9	5.4±2.1	10.8±0.2	19.6±2.5‡
22:5(*n*-3)	1.3±0.2	0.9±0.3	1.1±0.2	0.7±0.1	—	0.5±0.5	—	—
22:6(*n*-3)	16.8±1.5	15.4±1.6	24.5±1.0	33.1±2.1‡	69.9±5.1	55.6±21.5	1.9±0.2	2.4±0.5
Total sats	19.4±0.6	19.4±4.3	23.5±0.9	21.0±0.7§	11.5±5.0	13.9±10.5	26.0±1.0	25.4±1.3
Total monos	4.2±0.3	4.4±2.0	12.7±0.5	10.1±0.5‡	3.2±1.1	8.9±3.9	7.5±1.6	8.6±3.3
Total PUFA	76.4±0.9	76.2±6.2	63.8±0.9	68.9±1.3‡	85.3±5.9	76.8±13.7	66.5±8.6	66.0±0.5

Values are means±s.d. of three cultures.

Significantly different from corresponding 20° value, $*p \leq 0.001$; $\dagger p \leq 0.002$; $\ddagger p \leq 0.01$; $\S p \leq 0.02$; $\| p \leq 0.05$.

PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CL, cardiolipin; SL, sulpholipid; sats, saturates; monos, monoenes; PUFA, polyunsaturated fatty acids.

PE was only a minor component of the *C. salina* lipid. Although this phospholipid is a common component of lipid from eukaryotic algae, it was not detected previously in another unspecified *Cryptomonas* species [13]. Low contents of PE may be a characteristic of the Cryptomonad group of algae in general.

Detailed analyses of the lipid composition of microalgae have mostly been carried out with freshwater species. In such species, 18:3(*n*-3) is usually the predominant PUFA and occurs in both phospholipids and galactolipids. As a typical marine species, *C. salina* contains C_{20} and C_{22} PUFA in addition to C_{18} PUFA. A very noticeable feature of the *C. salina* lipid was that the galactolipids were characterized by high contents of C_{18} PUFA whereas 20:5(*n*-3) and 22:6(*n*-3) were located almost exclusively in the phospholipids. Although a similar situation also occurs in *Euglena gracilis*, the distribution pattern is not universal since the galactolipids of other marine microalgae such as *Porphyridium cruentum* are known to be rich in C_{20}, and almost devoid of C_{18}, PUFA [14].

The presence of both C_{18} and C_{20} PUFA in the PG + CL + SL fraction was evident, but nothing can be concluded from the present data as to the fatty acid composition of the three individual classes. The specific localisation of 16:1(*n*-13) *trans* in this fraction is in keeping with this fatty acid being a component of PG in photosynthetic cells [10, 14].

The component which co-chromatographed with PS and PI was designated PC_2 purely on the basis of its positive reaction with the specific stain used for the detection of PC. An earlier study of lipids in a Cryptomonad species also found a polar lipid with similar staining characteristic accounted for a significant proportion of the total polar lipids [13]. The exact nature of this lipid in Cryptomonads remains to be established.

The observed increase in lipid content of *C. salina* cultured at low temperature contrasts with previous reports that the lipid content of *Chlorella* species of microalgae does not vary to any great extent with culture temperatures in the range 14 to 28° [6, 7]. However, the temperature of 8° studied here is considerably lower than that examined in previous studies with microalgae [4–7]. The accumulation of lipid by *Chlorella emersonii* grown under conditions of suboptimal nutrient concentrations has been shown to render the algae more resistant to cellular damage caused by freezing and thawing [15]. The increased lipid content of *C. salina* may likewise serve to protect against the damaging effects of low temperature. The decreased content of sterols at lower temperature is in keeping with cholesterol playing a role in the modulation of lipid fluidity in membranes [16].

Previous studies have demonstrated that the total lipid of microalgae subjected to a lowering of temperature exhibit an increase in the overall degree of unsaturation of total lipid [4–7]. An overall increase in unsaturation was only observed here for MGDG and the PS + PI + PC_2 fraction. Although temperature related changes were observed in the other lipid classes, these were such that an increased proportions of some PUFA were balanced by decreases in others. The specific decrease in 18:2(*n*-6) coupled with the increases in 18:4(*n*-3) which was apparent in all lipid fractions suggests that the desaturation of 18:2(*n*-6) to 18:4(*n*-3) via 18:3(*n*-3) is enhanced at lower temperatures. In higher plants and algae, the formation of 18:3 from 18:2 is known to involve 18:2 esterified in MGDG as substrate for Δ^{15} desaturation [1]. Nothing is known however of the mechanisms by which 18:4(*n*-3) and longer chain PUFA are formed in photosynthetic algae. The predominance of 18:3(*n*-3) and 18:4(*n*-3) in galactolipids of *C. salina* are in keeping with galactolipids being a substrate for the Δ^{15} and Δ^{6} desaturases necessary for their formation. The presence of higher proportions of 18:3(*n*-6) in galactolipids than phospholipids might indicate that this PUFA can function as an intermediate in the formation of 18:4(*n*-3). Thus, 18:2(*n*-6) formed by the desaturation of oleoyl-PC might be esterified to MGDG or DGDG and acted upon by a Δ^{6} desaturase to produce 18:3(*n*-6) followed by subsequent Δ^{15} desaturation to yield 18:4(*n*-3). The formation of 18:3(*n*-6) in *Boragio officinalis* is known to proceed via this mechanism [17] and it has been suggested that this pathway operates for the formation of 18:4(*n*-3) in *Chlamydomonas reinhardii* [18].

The increased desaturation observed in leaves during cold adaptation has been ascribed [19] to the increased solubility at low temperatures of oxygen, a necessary substrate for the desaturase system. More recently, it has been proposed that the desaturase activity in chloroplasts is increased at low temperatures by the galactosyl transferase and desaturase enzymes becoming more closely associated and allowing more immediate access to newly formed MGDG [20]. Such an effect would also favour the increased formation of 18:4(*n*-3) observed in galactolipids at low temperature in the present study.

The distinct differences in PUFA composition between the galactolipids and phospholipids suggests that separate pathways operate for the synthesis of their PUFA components. Thus, the synthesis of 18:3(*n*-3) and 18:4 (*n*-3) may involve galactolipids as substrates whereas the formation of 18:2(*n*-6), 20:4(*n*-6), 20:4(*n*-3), 20:5(*n*-3) and 22:6(*n*-3) may be associated with phospholipids. Ongoing studies with *C. salina* are aimed at establishing the involvements of galactolipids and phospholipids in the biosynthesis of specific long chain PUFA.

EXPERIMENTAL

Alga and culture conditions. Chroomonas salina (Wislouch), obtained from the Scottish Marine Biological Association (Oban, Scotland), was cultured axenically in the basal seawater medium of ref. [21] to which 0.25 M glycerol had been added. Cultures of initial cell density 0.86×10^{-5} cells/ml and total vol. 500 ml were set up by inoculation from a stock culture. In triplicate, cultures were maintained at either 20 or 8° in a New Brunswick environmental chamber under continuous white light of intensity 60 $\mu E/m^2$ sec. The two different groups of cultures were carried out sequentially using the same environmental chamber. Cultures were continuously aerated with sterile air and cell numbers were determined daily using an improved Neuber counting chamber. Cultures were allowed to grow until their colour began to change from brown to green indicating the onset of stationary phase [12]. Cells were then harvested by centrifugation and freeze-dried as described previously [8].

Lipid analyses. Total lipid was extracted from the freeze-dried cells as described previously [8]. For the estimation of lipid classes, total lipid was separated into component classes by HPTLC on 10 × 10 cm glass plates coated with silica gel G using a double development system in which the chromatogram was developed to half its maximum using MeOAc–*iso*-PrOH–$CHCl_3$–MeOH–0.25% KCl (25:25:25:10:9) as the solvent system and then developed fully using hexane–Et_2O–HOAc (40:10:1). The developed chromatograms were stained with the $Cu(OAc)_2$ reagent of ref. [22] and the sepd lipid classes identified by comparison of their R_f values with those of authentic standards. The lipid classes were then quantitated by densitometry using a Shimadzu CS 9000 dual wavelength flying spot scanner attached to a Shimadzu DR-13 data recorder. To confirm the identification of components, stains specific for glycolipids and individual phospholipids were also applied to developed chromatograms [23]. The R_f of sulpholipids in the HPTLC system used had been determined previously by growing a culture of another microalgal species in the presence of ^{35}S followed by autoradiography of the sepd lipid.

For analysis of their fatty acid compositions, lipid classes were separated by TLC using the solvent system described above and made visible by spraying developed chromatograms lightly with 2′,7′-dichlorofluorescein and viewing under UV light. Bands of adsorbent containing the required lipid classes were scraped from the glass plates and subjected directly to acid-catalysed transesterification to produce the Me ester derivatives of fatty acids present in lipid classes [23]. The FAMEs were analysed by GC with FID using a 50 m × 0.32 mm i.d. fused silica column coated with CP Wax 52CB. H_2 was used as carrier gas and sample application was by on-column injection. Oven temp. was programmed to rise from 50 to 225° during each analysis. Sepd components were identified by comparison with known standards and the unsaturated nature of components established when necessary by the re-analysis of samples after hydrogenation over PtO_2 as catalyst. The identification of 16:1(*n*-13) *trans* was confirmed by GC-MS using the conditions described elsewhere [24].

Acknowledgement—We thank Dr W. W. Christie (Hannah Research Institute, Ayr, Scotland) for confirmation of fatty acid identities by GC-MS.

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