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LIPID COMPOSITION DURING GROWTH OF MOTILE AND COCCOLITH FORMS OF *EMILIANIA HUXLEYI*

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Key Word Index—*Emiliania huxleyi*; Prymnesiophyceae; flagellate; coccolith; alkenone; polyunsaturated fatty acids.

Abstract—Flagellated and coccolith cell types of *Emiliania huxleyi* were grown axenically and the lipid class and fatty acid compositions of the main polar lipid classes examined throughout the growth cycle. The lipid compositions of the two cell types were similar with only slight differences noted, the coccolith form tending to have higher levels of neutral lipids than the flagellate. Methyl and ethyl ketones were present in both cell types. The proportion of phospholipids and glycolipids increased during the log-phase, while neutral lipids (free fatty acids, triacylglycerols, ketones and hydrocarbons) achieved their highest levels in the late stationary-phase. The polar lipids of both cell types were very rich in polyunsaturated fatty acids (PUFA). In sulphoquinovosylglyercol + phosphatidylethanolamine, 18:3*n*-3 and 18:4*n*-3 were the predominant fatty acids totalling 42.4–55.7%, while 18:5*n*-3 (33.1–79.2%) was the main fatty acid in digalactosyldiacylglycerols. This fatty acid was also predominant in monogalactosydiacylglycerols (36.6–57.7%), with lesser amounts of 18:4*n*-3 and 22:6*n*-3. Phosphatidylcholine was dominated by two molecular species, 22:6*n*-3/22:6*n*-3 and 14:0/22:6*n*-3, accounting for 25.7–33.7 and 42.0–58.3%, respectively. Changes in PUFA composition over the growth cycle were small. The C_{18} PUFA associated with thylakoid glycolipids tended to increase during the log-phase and decreased in the late stationary-phase while 22:6*n*-3 peaked in the late stationary-phase. The results are discussed in relation to the ecology of this alga.

INTRODUCTION

The neutral lipids of the prymnesiophyte alga, *Emiliania huxleyi* have attracted considerable attention over the last 25 years or so since the discovery of very long-chain alkenones and alkenoates in marine sediments, which largely originate from this organism [1]. The finding that the number of double bonds in these compounds is empirically related to the growth temperature of the alga has led to them being used as biogeochemical indices of paleotemperatures [2–4]. *Emiliania huxleyi* is a very widespread and abundant alga being found in inshore and oceanic waters; blooms can cover areas of hundreds of square kilometres [5, 6]. The role of the coccolith form of *E. huxleyi* in carbon cycling in the oceans has also been the subject of many studies, given the global distribution and abundance of this species [6, 7].

The life cycle of *E. huxleyi*, particularly its overwintering strategy, are not understood [7]. The alga can exist as three possible cell types, a coccolith-forming cell (the C-cell), a completely naked cell (the N-cell) and a scaly motile cell (the S-cell) [8]. Each cell type is capable of independent vegetative propagation and can be grown in pure culture [8]. The environmental and physiological signals controlling the interconversions of these cell types remain unknown. In pure cultures of any of the three cell types, representatives of one of the other cell types may arise spontaneously. The flagellated S-cell contains half the amount of DNA found in the C-cell [9] and may be a gamete involved in sexual reproduction [8]. It has been suggested [10] that the long-chain ketones in *E. huxleyi* are a buoyancy mechanism for the heavy coccolith form.

The polar lipids from prymnesiophyte and dinomastigote algae are also of interest since they contain large amounts of the two most polyunsaturated fatty acids found in nature, docosahexaenoic acid (22:6*n*-3) and octadecapentaenoic acid (18:5*n*-3) [11, 12]. In the present study, we determined the lipid class composition, the fatty acid composition of the main glycolipid classes and the molecular species composition of phosphatidylcholine (PC) throughout the growth cycle in both flagellate and coccolith forms of *E. huxleyi* in order to gain a better understanding of the role of lipids in the ecology and physiology of this organism.

RESULTS

Cultures of flagellate and coccolith forms of *E. huxleyi* both grew rapidly (doubling time 13.8 and 12.5 hr,

respectively) and were moving into stationary-phase after seven and five days, by which time, cell density had increased 40- and 25-fold, respectively. Samples of the inoculum (stationary-phase cells, day −1), log-phase cells (days 2, 4 and 7) and stationary-phase cells (days 9, 12, 16 and 18) were taken for lipid analysis.

The lipid class composition of both the flagellated and coccolith cell types were similar (Tables 1 and 2, respectively) and changes during the growth cycle were generally small. In the flagellated cell type, amounts of PC increased slightly during the log-phase and into early stationary phase, before falling in the late stationary-phase (Table 1).

Table 1. Lipid class composition of the flagellated type of *E. huxleyi* with growth (wt% of total lipid)

	Day						
Lipid class	−1	2	4	7	9	12	18
Lysophosphatidylcholine	1.0 ± 0.2	tr*	tr	tr	tr	0.9 ± 0.3	0.9 ± 0.2
Phosphatidylcholine	12.2 ± 1.4	15.0 ± 1.1	16.2 ± 1.8	13.3 ± 1.7	15.5 ± 3.1	15.1 ± 3.6	11.7 ± 3.3
Phosphatidylinositol	tr	tr	tr	tr	0.6 ± 0.3	0.6 ± 0.2	tr
Phosphatidylgylcerol	1.3 ± 0.3	1.2 ± 0.1	1.4 ± 0.7	1.5 ± 0.5	1.9 ± 0.5	1.5 ± 0.2	1.6 ± 0.4
Sulphoquinovosylglycerol + phosphatidylethanolamine	7.7 ± 1.8	8.9 ± 1.8	7.8 ± 1.1	7.0 ± 0.8	8.9 ± 2.0	8.0 ± 0.7	5.4 ± 0.9
Digalactosyldiacylglycerols	4.7 ± 1.1	6.6 ± 1.0	6.8 ± 1.6	5.1 ± 0.6	6.4 ± 1.2	4.6 ± 0.6	4.0 ± 0.4
Monogalactosyldiacylglycerols + pigment 1	13.5 ± 2.3	20.8 ± 1.8	20.1 ± 0.6	16.5 ± 0.6	18.4 ± 2.3	16.4 ± 1.7	12.2 ± 2.8
Pigment 2	17.6 ± 2.8	17.2 ± 1.8	19.9 ± 2.5	20.7 ± 4.1	20.4 ± 4.9	20.9 ± 5.2	15.1 ± 6.3
Sterols	2.9 ± 0.6	1.7 ± 0.6	2.3 ± 0.2	3.6 ± 0.9	2.8 ± 0.6	2.3 ± 0.5	1.8 ± 0.9
Free fatty acids	2.0 ± 0.3	1.5 ± 0.3	1.8 ± 0.3	4.1 ± 2.2	1.5 ± 0.4	1.4 ± 0.1	1.7 ± 0.3
Triacylglycerols	2.1 ± 0.7	3.3 ± 1.6	1.5 ± 1.1	1.7 ± 0.5	0.6 ± 0.3	0.8 ± 0.3	4.1 ± 1.0
Methyl ketones	10.7 ± 0.4	9.7 ± 3.0	9.6 ± 3.7	8.4 ± 1.7	7.3 ± 3.2	10.0 ± 2.7	17.6 ± 4.8
Ethyl ketones	14.0 ± 2.8	4.4 ± 2.2	5.5 ± 3.2	7.4 ± 1.5	7.1 ± 4.1	11.3 ± 4.3	13.6 ± 6.7
Sterol esters	3.3 ± 0.8	3.3 ± 2.1	2.4 ± 1.4	4.2 ± 1.6	2.7 ± 1.8	1.9 ± 0.4	1.1 ± 0.6
Hydrocarbons	6.8 ± 3.1	3.5 ± 2.1	2.7 ± 2.4	5.7 ± 3.0	3.6 ± 2.1	5.2 ± 2.8	8.9 ± 3.9

Mean ± 1 SD, $n = 3$.
*Trace (= < 0.4%).
Pigment 1 = carotenoids; pigment 2 = chlorophylls.
Day −1 is the stationary phase culture used as inoculum.

Table 2. Lipid class composition of the coccolith type of *E. huxleyi* with growth (wt% of total lipid)

	Day						
Lipid class	−1	2	4	7	9	12	18
Lysophosphatidylcholine	tr*	0.4 ± 0.1	0.5 ± 0.1	tr	tr	tr	tr
Phosphatidylcholine	14.7	11.9 ± 0.1	11.5 ± 0.2	13.9 ± 1.7	15.5	14.6 ± 1.1	9.6 ± 1.9
Phosphatidylinositol	tr	tr	tr	tr	tr	tr	tr
Phosphatidylglycerol	4.3	2.4 ± 0.4	3.0 ± 0.3	3.4 ± 0.5	4.3	3.5 ± 0.9	1.3 ± 0.5
Sulphoquinovosylglycerol + phosphatidylethanolamine	9.5	6.0 ± 0.4	6.5 ± 0.7	7.9 ± 1.2	8.6	8.2 ± 0.9	4.6 ± 1.5
Digalactosyldiacylglycerols	7.0	6.5 ± 0.3	6.5 ± 0.3	6.2 ± 1.0	6.5	5.6 ± 0.7	3.9 ± 0.8
Monogalctosyldiacylglycerols + pigment 1	15.4	17.2 ± 2.2	16.8 ± 1.6	18.8 ± 0.9	18.5	18.2 ± 0.9	11.5 ± 1.3
Pigment 2	12.5	16.3 ± 0.3	18.0 ± 0.4	20.4 ± 4.5	17.6	20.0 ± 4.7	17.1 ± 4.4
Sterols	2.1	3.9 ± 0.3	3.6 ± 0.4	3.5 ± 0.4	4.7	4.1 ± 1.1	5.0 ± 0.5
Free fatty acids	4.3	5.8 ± 0.4	2.4 ± 0.0	1.3 ± 0.8	2.0	1.5 ± 0.7	10.0 ± 2.2
Triacylglycerols	tr	1.3 ± 0.9	tr	tr	tr	tr	3.3 ± 1.6
Methyl ketones	8.4	13.7 ± 2.1	12.5 ± 0.2	10.9 ± 2.2	9.2	8.9 ± 0.7	9.5 ± 1.4
Ethyl ketones	9.2	5.0 ± 1.8	6.0 ± 1.0	9.6 ± 3.0	7.3	8.1 ± 1.0	7.7 ± 1.4
Sterol esters	3.8	4.4 ± 0.6	2.5 ± 1.2	2.5 ± 0.7	3.5	2.9 ± 1.0	6.0 ± 3.1
Hydrocarbons	3.2	0.5 ± 0.5	9.4 ± 0.3	0.8 ± 0.7	1.8	2.1 ± 1.3	8.1 ± 3.6

Mean ± 1 SD, $n = 3$; only one determination for day-1 and day 9 samples.
*Trace (= < 0.4%).
Pigment 1 = carotenoids; pigment 2 = chlorophylls.

Amounts of sulphoquinovosylglycerol (SQDG) + phosphatidylethanolamine (PE), digalactosyldiacylglyercol (DGDG) and phosphatidylglycerol were relatively constant with a trend towards smaller amounts of SQDG and DGDG in the late stationary-phase, although the low amounts on day 7 confused the trend. Monogalactosyldiacylglyercol (MGDG) + pigment 1 (mainly carotenoids) and pigment 2 (chlorophylls) increased during the log-phase (Table 1). In the neutral lipids, methyl and ethyl ketones, hydrocarbons (HC) and triacylglycerols (TAG) showed the highest proportions in the late stationary-phase (days 1 and 18), although some classes again showed relatively high levels at intermediate times, e.g. day 2, TAG; day 7 HC (Table 1). The free fatty acid (FFA) fraction (including very long-chain alkenoates > C30) was also elevated in the day 7 sample. Sterols showed little change, while sterol esters (SE) decreased during growth (Table 1).

Most of the above trends were also apparent in the coccolith cell type, although here the inoculum (day −1) contained higher levels of all the main phospho- and glyco-lipid classes, and lower levels of neutral lipids (Table 2) than those of the initial flagellate culture. The amounts of ketones, SE and sterols changed little with growth (Table 2). By day 18, there was an accumulation of neutral lipids totalling 49.6%, with sterol, FFA, TAG, SE and HC (apart from a high value for HC on day 4) reaching their highest amounts (Table 2).

Differences in the lipid class composition between the two cultures were slight. The coccolith form contained slightly higher proportions of ketones during the log-phase (day 2–day 7) than the flagellate but, by day 18, the flagellate form contained almost twice as many ketones as did the coccolith form. The late stationary-phase culture of the coccolith form contained larger amounts of sterol, SE and FFA than did the flagellate (Tables 1 and 2).

The fatty acid composition of the three glycolipid classes were similar in both the flagellate and coccolith forms of *E. huxleyi* (Tables 3 and 4, respectively). There were great similarities in the fatty acid composition of MGDG and DGDG throughout the growth phase (Tables 3 and 4); 18:5*n*-3 dominated both galactolipids, amounts falling sharply at the start of the log-phase before increasing to over 60% in stationary-phase cultures. DGDG from the coccolith form contained especially large amounts of this fatty acid, with over 80% in stationary-phase cultures (Table 4). The much smaller amounts of 18:4*n*-3 and 18:3*n*-3 tended to increase during the log-phase (Tables 3 and 4), while amounts of 22:6*n*-3 fell sharply from the stationary-phase inoculum to log-phase cultures and remained low into the stationary-phase. In the coccolith form, there was more 18:4*n*-3 in MGDG than in DGDG (Table 4). This difference was less clear cut in the flagellate form (Table 3). Palmitic acid increased in ΔGΔG from log-phase cultures then decreased during stationary-phase in the flagellate form

Table 3. Fatty acid compositions of glycolipid classes of the flagellate type of *E. huxleyi* with growth (wt% major fatty acids)

Class	Fatty acids	Day					
		−1	2	4	7	12	18
SQDG/PE	14:0	18.0	15.9	15.8	12.0	15.4	17.2
	16:0	10.1	10.9	14.7	15.5	10.6	10.4
	18:1	7.0	6.7	4.4	9.9	7.0	8.7
	18:3*n*-3	21.5	26.7	25.2	22.9	24.0	23.3
	18:4*n*-3	23.9	16.6	18.8	19.5	23.4	20.2
	18:5*n*-3	2.1	1.2	1.6	0.8	1.9	1.0
	22:6*n*-3	6.3	6.9	4.7	2.6	4.3	4.3
DGDG	14:0	10.2	8.2	10.9	11.0	10.8	13.0
	16:0	6.4	12.3	17.2	11.4	9.9	10.4
	18:1	6.9	16.4	10.0	10.3	9.6	20.6
	18:3*n*-3	3.2	4.0	2.3	2.8	2.8	7.4
	18:4*n*-3	2.5	4.8	2.5	2.5	2.1	3.0
	18:5*n*-3	62.1	37.3	41.9	48.5	55.3	33.1
	22:6*n*-3	3.1	1.6	1.7	0.9	1.5	1.9
MGDG	14:0	11.2	10.8	6.3	9.1	10.7	6.9
	16:0	6.2	5.0	4.5	7.2	9.5	5.0
	18:1	12.8	14.2	11.3	14.0	12.7	18.7
	18:3*n*-3	1.9	2.8	2.5	2.0	2.8	5.7
	18:4*n*-3	8.6	16.3	13.9	10.2	8.1	9.3
	18:5*n*-3	44.6	40.9	52.5	48.6	43.1	41.6
	22:6*n*-3	8.2	3.8	1.7	3.6	4.1	6.0

18:1 includes *n*-9 and *n*-7 isomers. The following fatty acids were present also in small amounts: 15:0, 16:1*n*-9 and *n*-7, 18:0, 18:2*n*-6, 20:5*n*-3 and 22:5*n*-3.

Table 4. Fatty acid compositions of glycolipid classes of the coccolith type of *E. huxleyi* with growth (wt% major fatty acids)

Class	Fatty acids	Day					
		−1	2	4	7	12	18
SQDG/PE	14:0	21.6	17.4	20.8	16.0	18.8	18.5
	16:0	7.0	10.9	11.1	9.6	10.9	10.7
	18:1	1.8	5.4	3.0	2.1	2.8	3.3
	18:3*n*-3	17.1	27.1	30.1	27.1	21.6	21.4
	18:4*n*-3	28.1	18.0	21.9	28.6	26.5	24.5
	18:5*n*-3	5.7	1.9	1.4	1.8	2.4	2.0
	22:6*n*-3	11.1	6.1	4.5	4.8	4.5	7.4
DGDG	14:0	5.4	10.2	7.0	6.6	5.1	7.4
	16:0	2.7	8.4	6.9	8.0	10.3	9.7
	18:1	1.5	10.9	6.4	6.2	4.1	7.9
	18:3*n*-3	1.2	3.4	2.2	1.6	1.3	2.9
	18:4*n*-3	1.6	6.5	4.0	2.1	0.9	2.8
	18:5*n*-3	79.2	46.9	62.2	65.1	68.4	54.0
	22:6*n*-3	6.6	1.7	1.8	2.5	1.5	4.6
MGDG	14:0	13.9	9.9	10.0	6.0	9.9	12.0
	16:0	3.0	3.7	5.3	4.5	7.3	7.5
	18:1	5.2	11.7	6.9	6.2	7.4	8.4
	18:3*n*-3	1.5	2.1	1.3	1.1	2.7	2.1
	18:4*n*-3	9.1	17.9	19.3	11.6	10.4	11.1
	18:5*n*-3	46.1	36.6	47.5	57.7	43.8	41.6
	22:6*n*-3	16.7	9.1	2.9	7.3	9.9	8.8

18:1 includes *n*-9 and *n*-7 isomers. The following fatty acids were present also in small amounts: 15:0, 16:1*n*-9 and *n*-7, 18:0, 18:2*n*-6, 20:5*n*-3 and 22:5*n*-3.

Table 5. Molecular species compositions of PC from flagellated and coccolith types of *E. huxleyi* (mol %)

		Day							
		−1	2	4	7	9	12	16	18
Flagellate	22:6/22:6	32.4 ± 0.0	25.8 ± 1.4	25.7 ± 0.3	31.4 ± 1.7	30.6 ± 2.5	29.7 ± 1.1	32.0 ± 0.1	32.0 ± 2.4
	14:0/22:6	42.8 ± 0.8	42.0 ± 0.8	50.1 ± 0.4	47.6 ± 0.2	48.3 ± 1.3	45.9 ± 0.2	44.8 ± 0.4	46.7 ± 1.9
	18:1/22:6	6.4 ± 0.1	6.4 ± 0.2	6.0 ± 0.0	4.6 ± 0.2	5.1 ± 0.4	5.0 ± 0.0	4.9 ± 0.1	5.2 ± 0.0
	16:0/22:6	9.6 ± 0.3	10.3 ± 0.4	7.3 ± 0.1	6.9 ± 0.2	8.0 ± 0.2	7.2 ± 0.1	6.9 ± 0.1	8.2 ± 0.2
	others	8.8	15.5	10.9	9.5	8.0	12.2	11.4	7.9
Coccolith	22:6/22:6	33.7 ± 0.1	26.7 ± 1.0	26.7 ± 0.7	28.2 ± 3.0	29.6 ± 1.8	26.4 ± 1.2	32.0 ± 0.6	29.4 ± 0.3
	14:0/22:6	47.0 ± 0.0	50.4 ± 0.3	57.0 ± 0.8	58.3 ± 1.8	55.4 ± 1.6	55.9 ± 0.9	52.3 ± 0.6	55.5 ± 0.8
	18:1/22:6	4.4 ± 0.0	5.7 ± 0.0	4.5 ± 0.5	3.0 ± 0.4	4.0 ± 0.1	3.5 ± 0.3	3.5 ± 0.1	3.7 ± 0.0
	16:0/22:6	6.6 ± 0.0	7.7 ± 0.1	3.6 ± 0.1	3.1 ± 0.1	5.1 ± 0.2	5.0 ± 0.0	3.8 ± 0.1	5.1 ± 0.1
	others	8.3	9.5	8.2	7.4	5.9	9.2	8.4	6.1

Mean of two determinations ± range.

(Table 3) but remained high in the coccolith form (Table 4). Levels of 14:0 generally remained unchanged in both cultures.

SQDG and PE were not separated by the chromatographic system used. Algae tend to have little or no PE [13], so that the band measured here was probably mainly composed of SQDG. The fatty acid composition suggests this was the case, in that it was dominated by 18:3*n*-3 and 18:4*n*-3, which are characteristic of glycolipids rather than phospholipids. The amounts of 18:3*n*-3 increased slightly during the log-phase, while the converse was true for 18:4*n*-3 (Tables 3 and 4). Amounts of 18:5*n*-3 were low in SQDG and also fell during growth, as did the amounts of 22:6*n*-3. There was a decrease in 14:0 during the log-phase and increases in 16:0 from a lower starting level in the inoculum (Tables 3 and 4).

The very large amounts (> 60%) of 22:6*n*-3 in PC from both cell types (data not shown) indicated that di22:6*n*-3 molecular species must be present. This was confirmed by molecular species analysis of PC which showed 22:6*n*-3-containing molecular species as the four largest components, dominated by 14:0/22:6*n*-3 and 22:6*n*-3/22:6*n*-3 (Table 5). Together, these two molecular

species comprised between 67.8 and 86.5% of PC in all samples. In both the flagellate and coccolith forms, there was a fall of 6–7% in di22:6*n*-3 PC from the initial stationary-phase cultures to early log-phase cultures before slight increases back towards *ca* 30% in stationary-phase cultures (Table 5). There was no difference in di22:6*n*-3 PC content between the two forms. The major molecular species, 14:0/22:6*n*-3, increased from the inoculum to early log-phase cultures in both forms, then remained relatively constant, while the amounts of 18:1/22:6*n*-3 and 16:0/22:6*n*-3 fell from stationary- to early log-phase cultures, then remained fairly steady (Table 2). There was a trend of more 14:0/22:6*n*-3 PC in the coccolith form than the flagellate form (54.1 ± 3.6 vs 46.0 ± 2.8, mean over all times) and less 18:1/22:6*n*-3 (4.0 ± 0.8 vs 5.5 ± 0.7) and 16:0/22:6*n*-3 (5.0 ± 1.6 vs 8.1 ± 1.3) (Table 5). This may be a function of cell density since the flagellate culture contained more cells at all sampling times.

DISCUSSION

The lipid class composition of the flagellate and coccolith cell types of *E. huxleyi* were similar. Glycolipids associated with photosynthesis increased during growth and fell slightly in late stationary-phase when cells were dying, while neutral lipid tended to accumulate in late stationary-phase. The presence of similar amounts of alkenones in the motile flagellate (no lith) and the coccolith cell type suggests these compounds are unlikely to be involved in bouyancy. The majority of algae use TAG as a storage lipid and the relatively low amounts in *E. huxleyi* might suggest that other neutral lipids, particularly the long-chain ketones, fulfil this role also. It was originally thought that, since these compounds are used as biogeochemical markers, they were resistant to degradation, but it is now known that they are degraded substantially before reaching the sediments [14]. The physiological role, biosynthesis and degradation of these unusual lipids deserves further attention.

The fatty acid composition of the total lipid from *E. huxleyi* was relatively simple being dominated by four polyunsaturated fatty acids (PUFA), 18:3*n*-3, 18:4*n*-3, 18:5*n*-3 and 22:6*n*-3. The saturated fatty acids (SFA) 14:0 and 16:0, were the other main constituents, with smaller amounts of 16:1*n*-9 and *n*-7, 18:0, 18:1*n*-9 and *n*-7, 18:2*n*-6, 20:5*n*-3 and 22:5*n*-3, confirming earlier studies on the coccolith form [15]. There was a large selectivity among the PUFA for different lipid classes with 22:6*n*-3 present predominantly in phospholipid (PC) and the C_{18} PUFA present overwhelmingly in the glycolipids. Molecular species containing 22:6*n*-3 dominated PC.

In PC from the non-photosynthetic dinomastigote alga *Crypthecodinium cohnii*, di22:6*n*-3 comprised 23.5–25.4% and was independent of growth temperature [16]. The other dominant molecular species were 14:0/22:6*n*-3 (28.7–35.7%) and 16:0/22:6*n*-3 (22.3–24.3%), these three molecules together accounting for 75–82% of the PC [16]. PC from *E. huxleyi* contained more di22:6*n*-3 and 14:0/22:6*n*-3 and less 16:0/22:6*n*-3 than did PC from *C. cohnii*, but overall the composition of PC from these two algae was remarkably similar. PC is the main membrane phospholipid in algae, being present in all membranes, except the photosynthetic thylakoid membranes. Whether di22:6*n*-3 PC is localized specifically in the plasma membrane or in internal membranes remains to be elucidated, as does the functional consequences of having such a restricted range of phospholipid molecular species in cellular membranes.

Phosphoacylglycerols containing PUFA on both the *sn*-1 and *sn*-2 positions of glycerol have a very restricted distribution in cell membranes from animals, the normal structure being a SFA or monounsaturated fatty acid (MUFA) on *sn*-1 and a MUFA or PUFA on *sn*-2. Di22:6*n*-3 molecular species of PE and phosphatidylserine (PS) are present in neural tissue, especially rod outer segment membranes of terrestrial vertebrates [17] and are particularly abundant in marine fish, accounting for 71.8% of PE and 59.7% of PS in the retina of cod [18]. In rod outer segments, di22:6*n*-3 phospholipids are thought to be essential in providing the correct liquid-crystalline matrix in which rhodopsin can function [19]. Given the large amounts of di22:6*n*-3 phospholipid found in rod cells, it is of interest to note that the vertebrate rod cell may have evolved from a flagellated cell [20] and that a rhodopsin photoreceptor has been identified in the flagellated alga, *Euglena gracilis* [21]. The role of di22:6*n*-3 PC in marine algae remains to be elucidated.

The glycolipids MGDG, DGDG and SQDG are found almost exclusively in the thylakoid membranes of plants and photosynthetic algae. They have a very characteristic fatty acid composition usually dominated by C_{16} and C_{18} PUFA with smaller amounts of 16:0, 16:1 and 18:1 fatty acids. The PUFA composition is species-dependent [22]. Prymnesiophyte and dinomastigote algae are the only organisms known to contain 18:5*n*-3 [12]. Given the specificity of C_{18} PUFA in glycolipids rather than phospholipids in other algae, it was expected that the 18:5*n*-3 would be localized in the glycolipids in *E. huxleyi*. Only trace amounts (< 1%) were present in the PC samples. However, the selective location of 18:5*n*-3 in DGDG, especially in the coccolith form, was particularly impressive.

Why does this organism utilize 18:5*n*-3 rather than 18:4*n*-3 or 18:3*n*-3 in the galactolipids since it is energetically more expensive to synthesize, and what are the functional consequences for the cell? *E. huxleyi* exhibited a higher quantum efficiency and, therefore, higher photosynthetic rate than the diatom, *Chaetoceros gracile* [23]. This was related to a more efficient use of light between 500 and 550 nm due to the unusual pigment 19′-hexanoylfucoxanthin [24], which can contribute 89% of the carotenoids in this species [25]. Perhaps the large amount of 18:5*n*-3 in thylakoid galactolipids is in some way linked to the presence of this pigment and the resulting greater photosynthetic efficiency, which may explain why *E. huxleyi* is such a dominant component of the phytoplankton.

The biosynthesis of 18:5*n*-3 is also of interest. There is now good evidence in mammals that 22:6*n*-3 is not synthesized from 22:5*n*-3 by Δ-4 desaturation, but by elongation of 22:5*n*-3 to 24:5*n*-3 and Δ-6 desaturation of the latter to form 24:6*n*-3, which is then chain-shortened by β-oxidation in peroxisomes to 22:6*n*-3 [26]. By analogy, 18:5*n*-3 could be formed by chain-shortening of 20:5*n*-3. The 'classical' pathway for the biosynthesis of 18:5*n*-3 would require a Δ-3 desaturase operating on 18:4*n*-3. The variant pathway described by Voss *et al.* [26] has not so far been directly demonstrated in any alga. However, if this biosynthetic route is operative in *E. huxleyi*, the chain-shortening must be very efficient and selective, since all of the 24:6*n*-3 formed is converted into 22:6*n*-3 and almost all the 20:5*n*-3 into 18:5*n*-3. The largest proportions of 20:5*n*-3 and 22:5*n*-3 were present in the SQDG/PE fraction, suggesting that this lipid(s) could be a biosynthetic intermediate.

The close similarity of both the lipid class and fatty acid composition of the coccolith and flagellate cell forms suggests that the lipid composition of *E. huxleyi* is determined by genotype rather than phenotype. However, a number of interesting questions remain unresolved. The biological role, synthesis and degradation of the alkenones remain to be elucidated while the selective synthesis of a narrow spectrum of fatty acids indicates close control of *de novo* synthesis. The large amounts of 18:5*n*-3 and 22:6*n*-3 indicate a very active final step in PUFA biosynthesis, whether by conventional Δ-4 desaturation or by the alternative pathway. Finally, the functional consequences of having a very narrow range of fatty acids and, therefore, molecular species in membrane lipids remains to be elucidated.

EXPERIMENTAL

Algal cultures. C-cell and S-cell forms of *E. huxleyi* (Lohm.) Hay et Mohler, strain DWN 61/81/5, were obtained from the Plymouth Marine Laboratory collection. Isolates were grown at 15° in F/2 medium under a 12 hr light-dark cycle (100 μE sec^{-1}) using 75 W fluorescent tubes. Cell numbers were monitored daily and samples harvested for lipid analysis at intervals during the growth cycle by filtering on to ashed glass-fibre filters, and stored at −40° in $CHCl_3$–*iso*-PrOH (2:1). Samples of each culture were examined at the end of the expt to check that the cell type had not changed.

Lipid analysis. A Folch extract [27] of total lipid was prepd and the lipid class composition determined by double-development HPTLC as described in ref. [28]. Briefly, 10 μg of total lipid was chromatographed on pre-run HPTLC plates with MeOAc–*iso*-PrOH–$CHCl_3$–MeOH–0.25% aq. KCl (25:25:25:10:9) to 3.5 cm to separate polar lipids, air-dried, then chromatographed in hexane–Et_2O–HOAc (90:10:1) to 9.5 cm to separate neutral lipids. Lipids were visualized with CuOAC–H_3PO_4, charred and quantitated by scanning densitometry [28].

Polar lipid classes were sepd by HPTLC in the polar solvent system described above. Fatty acids were identified by GC of the corresponding Me esters on a BP20 fused-silica capillary column (50 m × 0.32 mm i.d.) with H_2 as carrier gas [29]. Identities of fatty acids were confirmed by GC-MS analysis of the corresponding picolinyl esters. The GC was fitted with a fused-silica capillary column (DB5-MS, 15 m × 0.25 mm i.d.) and He was used as carrier gas. 9-Anthroyl derivatives of PC were prepd and sepd into molecular species as described previously [30].

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