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LIPID COMPOSITION OF *EUGLENA GRACILIS* IN RELATION TO CARBON–NITROGEN BALANCE

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Abstract — The lipid class and fatty acid compositions of *Euglena gracilis* were analysed after growth under various conditions of autotrophy or photoheterotrophy, in order to identify the contribution of lactate (a carbon source) and ammonium phosphate (a nitrogen source) to the metabolism of these compounds. When ammonium and lactate were both present in the medium, in concentrations that allowed growth, the lipid composition of algae appeared to be independent of the ammonium concentration. The effects of increasing ammonium phosphate concentration on lipid metabolism were observed only when lactate was depleted. With increasing nitrogen concentration in the medium, an increase in the content of galactolipids rich in polyunsaturated C_{16} and C_{18} fatty acids (FA) was noted, as well as an increase in the monogalactosyldiacylglycerol : digalactosyldiacylglycerol ratio; these changes could be related to an increase in the number of the stacked lamellae present in chloroplasts. Nitrogen concentration had no influence on the C_{12} and C_{14} FA but induced a loss of C_{22} FA. In the absence of ammonium phosphate in the culture medium, increasing lactate concentrations were accompanied by a decrease in all plastid lipids, whereas the content of storage lipids (enriched in 14 : 0 and 16 : 0) increased. Partial inhibition of the polydesaturation of C_{18} FA was observed and was accompanied by an accumulation of 18 : 1.

INTRODUCTION

Changes in the lipid composition of higher plants and algae often occur as a result of variations in environmental or culture conditions [1, 2]. The effects of nitrogen concentration in the culture medium, in particular, have been studied [3, 4]. In the presence of high nitrogen levels, the green algae, *Chlorella vulgaris*, *Scenedesmus obliquus* [5] and *Fritschiella tuberosa* [6] produced large amounts of polar lipids. At low nitrogen levels, the contents of neutral lipids, essentially triacylglycerols (TAG), increased. On the other hand, C_{14} fatty acid (FA) contents seemed independent of nitrogen concentration [7]. Some authors have mentioned the presence of C_{20} and C_{22} FA in species of the Chlorophyceae [5, 7, 8], but to the best of our knowledge, no study has ever been undertaken on the influence of nitrogen concentration on long-chain FA in algae.

Euglena gracilis, like other green algae, grows in photoautotrophic conditions; moreover, it is also able to grow in both heterotrophic or phototrophic conditions. Thus, it is a useful model system for testing carbon–nitrogen influences on lipid metabolism. *Euglena gracilis* can synthesize substantial amounts of polyenoic C_{20} and C_{22} FA [9], the amount of which was observed to increase in media containing sugar [9, 10], suggesting an influence of exogenous carbon upon the synthesis of these compounds. However, few data [9, 11] have shown an influence of exogenous carbon on lipid class and FA compositions of *Euglena*. Thus, systematic investigations on the effects of changing carbon and nitrogen concentrations on the lipid composition of this alga have still to be performed. In the present paper, we have analysed the lipid classes and the FA composition of *E. gracilis* cultivated under various conditions of autotrophy or heterotrophy.

RESULTS AND DISCUSSION

Euglena gracilis was grown under different conditions (Table 1). We started from three complete media, each one containing lactate (33 mM) and either 0.95, 1.5 or 3.8 mM of ammonium phosphate $(NH_4)_2HPO_4$). Nitrogen must be provided as ammonium because *Euglena* has no nitrate reductase; ammonium sulphate reduced growth. These media which produced photoorganotrophy without any restriction, and which allowed

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growth at the same rate whatever the $(NH_4)_2HPO_4$ concentration in the medium [12] will be called, hereafter, C + N + media. The exponential phase of growth could be observed in the three media at day 2. As cells used nutrients, three trophic conditions were obtained at stationary phase (days 4–7): (1) for algae initially cultivated on 1.5 mM $(NH_4)_2HPO_4$, lactate and nitrogen were both exhausted at the stationary phase (conditions of photoautotrophy with depletion of nitrogen: C − N − medium). Cell numbers reached 3.2×10^6 ml^{-1}; (2) for algae initially cultivated on 3.8 mM $(NH_4)_2HPO_4$, the medium at stationary phase was depleted in lactate but still contained nitrogen (conditions of photoautotrophy not limited in nitrogen: C − N + medium). Cell numbers reached 3.4×10^6 ml^{-1}; (3) for algae initially cultivated on 0.95 mM $(NH_4)_2HPO_4$, lactate was still present at stationary phase but nitrogen was exhausted (conditions of photoheterotrophy limited in nitrogen: C + N − medium). Cell numbers reached 1.2×10^6 ml^{-1}. Comparison between lipid compositions of algae at stationary phase in such different trophic conditions thus allowed us to evaluate the relative effects of ammonium and organic carbon on the synthesis of FA.

Quantitative evolution of FA content

The amounts of total FA of *Euglena* cells were clearly different at day 0 (Fig. 1). Indeed, inoculated cells were five-days-old and their metabolism had then taken on individual characteristics corresponding to the different nutritional conditions (Table 1).

During the exponential phase of growth (day 2, C + N + conditions) total FA contents reached *ca* 60 pg cell^{-1} (Fig. 1). The slight differences observed between different media were not significant. The total FA amount per cell was thus independent of $(NH_4)_2HPO_4$ concentration in the medium. Marked differences occurred only when nutrient limitation appeared (after day 4). When lactate and nitrogen were both lacking (C − N − medium), the FA content per cell was low (*ca* 40 pg cell^{-1}). The FA amount per cell was significantly higher (*ca* 50 pg cell^{-1}) when nitrogen persisted in the medium (C − N +). Thus, high levels of nitrogen in the medium enhanced FA synthesis by 25%, but its effect was not observed until the stationary phase was reached. In marked contrast, when lactate persisted and nitrogen was deficient (C + N − medium), the total FA content increased sharply; this content (*ca* 75 pg cell^{-1}) was twice or 1.5-fold higher, respectively, as compared to cells cultivated on C − N − and C − N + media. We conclude that *Euglena* cells used the exogenous carbon of the medium for the synthesis of FA which accumulated in non-dividing cells.

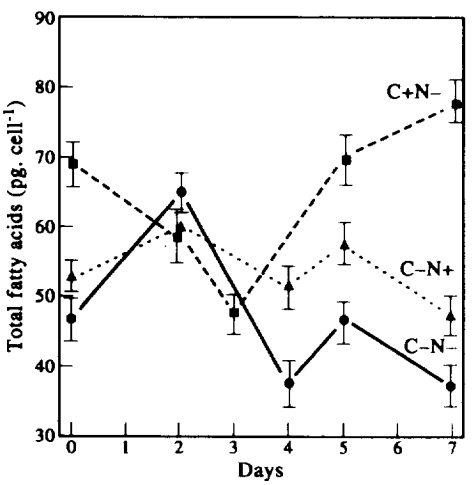

Fig. 1. Total fatty acid contents of *Euglena gracilis* cells during 7 days growth on three different media containing: (--■--) 0.95, (−●−) 1.5, or (---▲---) 3.8 ammonium phosphate. Trophic conditions detailed in Table 1. Values are means of five independent experiments, with standard deviations.

Changes in FA composition

Table 2 shows the fatty acids identified in the total lipids of *Euglena* cells cultivated on various media and at different ages. Under all conditions, C_{16} FA were dominant (31–42%), as found in several Bacillariophyceae and in some Dinophyceae [7, 13]. C_{20} FA were approximately as abundant as C_{18} FA (C_{20}, 18–25%; C_{18}, 17–30% of total FA weight). The predominance of C_{16} FA and the importance of C_{20} FA in *Euglena* has also been reported by Erwin and Block [14] and by Hulanika *et al.* [10] for cells cultivated under heterotrophic conditions; under photoorganotrophic or autotrophic conditions, these authors found a predominance of C_{18} FA.

When the medium contained both carbon and nitrogen (C + N + medium), saturated and monoenoic FA each represented *ca* 20% of the total FA, whilst polyunsaturated FA represented *ca* 60%.

When some nutrients became exhausted, marked differences between the total FA compositions of *Euglena* cells were observed in the different media at day 7. The presence of exogenous carbon in the medium (C + N −) enhanced by more than three times the percentages of saturated FA when compared with those of these acids found on C-media. The proportion of short-chain FA (12:0 and 14:0) increased markedly, whilst those of monoenoic FA (12:1 and 18:1) and those of polyunsaturated C_{20} FA increased less. On the other hand, when lactate persisted in the medium, the proportions of polyunsaturated C_{16}, C_{18} and C_{22} FA were reduced by two- or three-fold as compared with percentages found on C-media. Thus, a net decrease in the unsaturation index of the total FA was observed in the C + N − medium.

Comparison between the C − N − and C − N + media (Table 2) highlighted the influence of nitrogen, causing an increase in the percentages of 16:3, 16:4 and 18:3 at the expense of 16:0 ad 18:0. Under photoautotrophic conditions (C-media), the high proportions of 16:3, 16:4 and 18:3 found mainly in chloroplasts, are in agreement with the findings of other authors [5, 8, 10, 14–16] and with our previous observations concerning development of the plastidome [12].

Table 1. Effects of medium composition on growth of *Euglena gracilis* cells in culture. Stationary phase was reached in the absence or presence of either nitrogen or carbon*

Day	N	C	Cells (ml^{-1})	N	C	Cells (ml^{-1})	N	C	Cells (ml^{-1})
0	0.95 mM	33 mM	4×10^4	1.5 mM	33 mM	4×10^4	3.8 mM	33 mM	4×10^4
1	+	+	16×10^4	+	+	16×10^4	+	+	16×10^4
2	+	+	4×10^5	+	+	4×10^5	+	+	4×10^5
3	+	+	8×10^5	+	+	8×10^5	+	−	8×10^5
4	−	+	1×10^6	+		1.6×10^6	+	−	1.6×10^6
5	−	+	1.2×10^6	−		3.2×10^6	+	−	3.2×10^6
6	−	+	1.2×10^6	−		3.2×10^6	+	−	3.4×10^6
7	−	+	1.2×10^6	−		3.2×10^6	+	−	3.4×10^6

*N, $(NH_4)_2HPO_4$; C, lactate; +, present; −, absent.

Table 2. Fatty acid composition (% total fatty acid weight) of *Euglena gracilis* cells cultivated under different trophic conditions

Fatty acid class	Day 2 Medium			Day 7 Medium		
	C + N +			C + N −	C − N −	C − N +
	C (33 mM)			C (15 mM)	C(O)	C(O)
	N (0.95 mM)	N (1.5 mM)	N (3.8 mM)	N(O)	N(O)	N(1.5 mM)
Saturated						
12:0	0.7 (0.09)	1.2 (0.5)	1.0 (0.2)	1.6 (0.4)	0.2 (0.1)	0.2 (0.02)
14:0	9.0 (1.8)	10.7 (1.7)	10.5 (1.7)	18.5 (1.1)	4.2 (0.4)	3.3 (0.5)
16:0	9.9 (0.5)	9.7 (0.6)	9.7 (0.5)	16.8 (1.8)	7.2 (0.5)	5.6 (0.5)
18:0	1.4 (0.3)	1.1 (0.2)	1.2 (0.1)	1.3 (0.2)	0.4 (0.08)	0.2 (0.02)
Subtotal	21	22.7	22.4	38.2	12.0	9.3
Monounsaturated						
12:1	1.9 (0.2)	1.6 (0.1)	1.9 (0.8)	2.3 (0.5)	0.7 (0.3)	0.4 (0.1)
14:1	1.1 (0.3)	1.0 (0.1)	0.9 (0.08)	0.9 (0.3)	2.0 (1)	2.3 (1.0)
16:1*	8.7 (2.0)	9.2 (0.7)	7.9 (2.0)	6.3 (0.9)	8.8 (3.2)	10.8 (3.4)
18:1*	9.1 (0.6)	7.4 (1.4)	7.0 (0.8)	8.0 (1.4)	4.8 (0.8)	3.2 (0.3)
Subtotal	20.8	19.2	17.7	17.5	16.3	16.7
Polyunsaturated						
14:2	1.2 (0.4)	1.3 (0.2)	1.1 (0.3)	1.5 (0.4)	2.2 (0.3)	1.1 (0.2)
14:4	2.0 (0.8)	1.8 (0.2)	1.3 (0.06)	0.7 (0.1)	1.6 (0.3)	2.1 (0.2)
16:2	3.0 (0.3)	3.9 (1.0)	3.2 (0.4)	4.0 (0.2)	10.7 (2.1)	10.6 (1.8)
16:3	7.7 (0.7)	6.4 (0.3)	6.4 (0.2)	2.4 (0.9)	6.3 (1.1)	7.8 (0.3)
16:4	4.3 (0.7)	5.3 (0.6)	5.7 (0.4)	2.5 (1.1)	6.1 (0.9)	8.1 (0.8)
18:2	3.9 (0.2)	5.0 (1.2)	4.0 (0.9)	4.0 (0.4)	9.8 (1.6)	9.6 (1.4)
18:3	13.3 (1.0)	12.8 (1.9)	13.5 (1.1)	3.9 (0.7)	12.1 (1.1)	15.0 (1.4)
20:2	1.5 (0.2)	1.8 (0.2)	2.0 (0.4)	3.4 (1.1)	1.4 (0.9)	1.2 (0.1)
20:3	3.3 (0.1)	3.2 (0.2)	3.4 (0.2)	3.2 (0.6)	2.3 (0.4)	1.5 (0.1)
20:4	10.1 (0.9)	9.4 (0.9)	10.5 (1.3)	10.0 (1.2)	9.6 (0.8)	7.6 (0.9)
20:5	4.8 (0.3)	4.5 (0.4)	5.0 (0.5)	6.9 (0.2)	6.0 (0.5)	4.3 (0.3)
22:5 + 6	3.1 (0.4)	2.7 (0.4)	3.8 (0.5)	1.8 (0.2)	3.6 (1.1)	3.2 (0.7)
Subtotal	58.2	58.1	59.9	44.3	71.7	74.0
Total	100	100	100	100	100	100
Unsaturation index	2.1	2.1	2.2	1.6	2.4	2.5
Content (pg $cell^{-1}$)	59.1 (3.5)	55.4 (1.4)	62.5 (9.8)	76.8 (5.1)	39.0 (3.7)	49.1 (3.5)

**cis* + *trans*. Values are the means of five independent experiments: standard deviations in parentheses.

Changes in lipid classes

Under photoorganotrophic conditions, when nutrients were not limited (C + N + media, Fig. 2A), neutral lipids, phospho- and galactolipids each represented *ca* one-third of the total lipids whatever the initial ammonium concentration in the medium. However, depletion of nitrogen (C + N − medium, Fig. 2B) caused *Euglena* cells to accumulate neutral lipids (up to 72% of total lipids) composed essentially of TAG and wax esters (respectively, 58 and 36% of the neutral lipids). At the same time, phospholipids and galactolipids were poorly represented (less than 15% each). A redistribution of carbon from polar to neutral lipids had been observed previously in green algae [5, 8] at low nitrogen concentrations. Cells limited both in nitrogen and carbon (C − N − medium), which contained only 22% of neutral lipids but 50% of galactolipids, showed that the accumulation of neutral lipids and the degradation of galactolipids were not directly related to the absence of nitrogen, but resulted from the persistence of lactate in the medium. When lactate was missing (photoautotrophic C − N − and C − N + media, Fig. 2B), galactolipids formed the main lipid class (respectively, 50 and 68% of the total lipid weight).

Variations observed in galactolipid contents were mainly due to changes in monogalactosyldiacylglycerols (MGDG), the digalactosyldiacylglycerols (DGDG) being relatively stable (Table 3). Thus, during the transition from C + N + to C + N − media, a 50% decrease in the quantity of galactolipids occurred essentially due to variations of MGDG. Thus, the MGDG : DGDG ratio decreased from *ca* 2 to less than 1. This result can be correlated with a > 50% loss of chlorophylls [12]. At the same time, the phospholipid content of the algae decreased by half. These results indicated a general degradation of cell membranes, particularly of thylakoids, which is consistent with the diminution of the volume of the plastidome of algae observed previously in C + N − medium [12]. Fatty acids resulting from membrane lipid degradation could be redirected into storage lipids.

During the transition from photoorganotrophy to photoautotrophy (from C + N + to C − N − or C − N + media), MGDG increased only when nitrogen remained present in the medium. Thus, the MGDG : DGDG ratio reached 3, whereas it is normally *ca* 2 under photoautotrophic conditions [17]. At the same time, chlorophyll contents doubled and chloroplastic membranes were extensively developed [12]. Neutral lipids decreased by *ca* 50% and phospholipids diminished also independently of the concentration of nitrogen. As the medium became depleted in organic carbon, the carbon of catabolized neutral and phospholipids might be used to synthesize galactolipids.

From these results, we can conclude that the persistence of organic carbon in the growth medium depleted of nitrogen favoured the accumulation of storage lipids probably synthesized by an extra-chloroplastic pathway. On the contrary, the persistence of nitrogen in a medium depleted of lactate promoted the biosynthesis of galactolipids in chloroplasts.

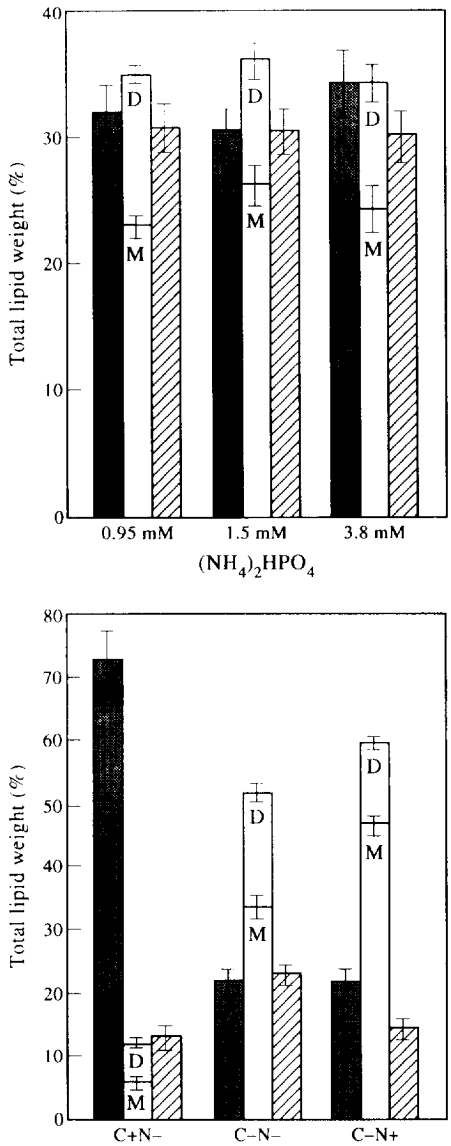

Fig. 2. Content of lipid classes (% total lipid weight) of *Euglena gracilis* cultivated on three different media. Data are means of three different experiments with standard deviations. (A) Lipid compositions, at day 2, on C + N + media with three ammonium phosphate concentrations and one lactate concentration (33 mM). (B) Lipid compositions at stationary phase under three different trophic conditions: C + N −, C − N − and C − N + media. (■); Neutral lipids; (□) galactolipids; D, DGDG; M, MGDG; (□) phospholipids.

Under all trophic growth conditions, phosphatidylcholine (PC) was the predominant phospholipid (*ca* 10% of total lipids, more than 50% of total phospholipids); phosphatidylglycerol (PG) represented only 3% of the total lipids. These results are in agreement with those of Oliveira *et al.* [18]. Phosphatidylinositol (PI) was not detected. During the evolution from the C + N + medium to other trophic conditions, PC decreased from 20 to 8% in the C + N − medium and from 20 to 15% in C − N − and C − N + media. Thus, the presence of

lactate in the medium apparently suppressed the biosynthesis of PC.

Fatty acid composition of main lipid classes

Analysis of the fatty acid distribution in the main lipid classes was performed on *Euglena* cells collected at the stationary phase on C + N −, C − N − and C − N + media (Table 4).

Under photoautotrophic conditions (C − N − and C − N + media), *cis* 16:1 was predominant (more than 50%) in the neutral lipids, whilst under photoorganotrophic conditions (C + N − medium), the proportion of this FA diminished five-fold in favour of 14:0 and 16:0.

Table 3. Quantities of lipid classes (pg cell^{-1}) in *Euglena gracilis* cells cultivated under different trophic conditions

	Medium			
	C + N + *	C + N − †	C − N − †	C − N + †
Neutral lipid	22.3 (2.1)	60.0 (5.4)	8.0 (0.6)	10.0 (0.9)
Monogalactosyldiacylglycerol	22.8 (1.8)	6.0 (0.7)	21.0 (1.9)	32.0 (2.7)
Digalactosyldiacylglycerol	11.8 (0.8)	9.8 (1.0)	12.0 (1.1)	11.0 (1.3)
Phospholipid	30.0 (2.7)	16.3 (1.5)	13.1 (0.9)	13.1 (1.2)
MGDG:DGDG	1.9	0.6	1.7	2.9

*Cells collected at day 2.
†cells collected at day 7. Values are the means of five independent experiments.

Table 4 Composition of fatty acids (% total fatty acids) in main lipid classes of *Euglena gracilis* cells cultivated on three different media*

		Medium		
Class	Fatty acids	C + N −	C − N −	C − N +
Neutral lipids				
	14:0	21.4 (1.7)	5.8 (0.5)	5.6 (0.6)
	14:1	1.8 (0.6)	4.4 (1.9)	6.8 (1.5)
	14:2	8.7 (0.2)	tr†	tr
	14:4	0.8 (0.1)	14.5 (0.4)	15.9 (0.1)
	16:0	15.8 (1.6)	5.3 (0.3)	2.7 (0.2)
	16:1 *cis*	11.0 (1.2)	51.2 (11)	60.5 (10)
	18:1 *cis*	6.3 (0.9)	5.8 (1.0)	2.5 (0.3)
	20:4	5.2 (0.7)	3.5 (0.3)	tr
	20:5	4.2 (0.2)	tr	tr
	Others	24.8	9.5	6.0
Monogalactosyldiacylglycerol				
	16:0	7.4 (0.8)	2.6 (0.1)	0.9 (0.08)
	16:1 *cis*	2.5 (0.3)	4.6 (1.1)	5.0 (1.5)
	16:2	18.4 (0.9)	24.9 (4.8)	20.6 (2.1)
	16:3	5.1 (0.4)	6.6 (1.1)	9.9 (0.04)
	16:4	11.0 (0.1)	11.9 (1.4)	13.6 (1.4)
	18:0	3.5 (0.6)	0.4 (0.07)	0.2 (0.02)
	18:1 *cis*	7.8 (0.9)	1.9 (0.2)	1.0 (0.1)
	18:2	16.5 (1.7)	20.1 (0.4)	15.9 (1.7)
	18:3	22.3 (3.1)	20.6 (1.9)	23.5 (2.3)
	20:4	3.8 (0.4)	0.9 (0.08)	0.7 (0.08)
	Others	1.7	5.5	8.7
Digalactosyldiacylglycerol				
	16:0	11.7 (1.1)	4.7 (0.3)	4.6 (0.4)
	16:2	22.4 (1.1)	26.0 (5.0)	21.0 (2.2)
	16:3	8.7 (0.7)	13.6 (1.6)	17.8 (2.1)
	18:1 *cis*	8.6 (1.3)	4.4 (0.8)	2.3 (1.2)
	18:2	18.9 (1.9)	21.9 (3.5)	19.9 (2.0)
	18:3	14.9 (2.8)	12.4 (1.2)	19.1 (1.8)
	20:4	5.3 (0.6)	3.2 (0.3)	1.8 (0.2)
	Others	9.5	13.8	13.5

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Table 4. *Continued*

Class	Fatty acids	Medium C + N −	C − N −	C − N +
Phosphatidylglycerol				
	16:0	10.1 (1.1)	8.7 (0.7)	28.2 (2.5)
	16:1 *trans*	32.1 (2.9)	33.3 (2.8)	14.2 (2.9)
	18:0	3.6 (0.6)	4.0 (0.07)	22.3 (0.2)
	18:1 *cis*	9.2 (1.0)	6.6 (1.1)	tr
	18:2	26.1 (2.7)	33.5 (4.6)	26.1 (2.7)
	18:3	5.1 (1.0)	7.2 (0.2)	7.6 (0.8)
	20:2	4.2 (0.2)	tr	tr
	20:4	8.4 (0.7)	2.2 (0.3)	tr
	Others	1.2	4.5	1.6
Phosphatidylcholine				
	14:0	2.1 (0.4)	3.9 (0.04)	7.1 (0.9)
	14:2	0.7 (0.2)	4.1 (0.5)	2.7 (0.4)
	16:0	16.3 (1.6)	12.6 (1.1)	10.0 (0.9)
	20:2	10.5 (2.0)	9.7 (0.8)	7.3 (0.6)
	20:3	5.6 (1.0)	2.2 (0.3)	tr
	20:4	14.2 (1.5)	20.1 (1.9)	28.5 (2.9)
	20:5	23.5 (0.7)	19.7 (1.8)	18.5 (1.8)
	Others	27.1	27.7	25.9
Phosphatidylethanolamine				
	14:0	3.2 (0.3)	3.9 (0.4)	5.3 (0.6)
	14:2	1.2 (0.04)	5.8 (0.6)	2.3 (0.4)
	16:0	28.6 (2.9)	14.7 (1.2)	9.1 (0.9)
	16:1 *cis*	2.8 (0.4)	1.1 (0.3)	2.9 (1.0)
	16:3	1.2 (0.5)	7.8 (0.9)	12.5 (0.6)
	18:1 *cis*	28.4 (2.9)	31.2 (0.6)	41.1 (0.4)
	18:2	3.2 (0.3)	2.5 (0.3)	4.8 (0.7)
	20:4	23.4 (2.4)	21.0 (2.6)	13.9 (1.5)
	20:5	3.8 (0.1)	3.3 (0.3)	3.0 (0.2)
	Others	4.2	8.7	5.1

*Values are the means of three independent experiments.
† tr, trace.

The polyunsaturated FA (PUFAs), 20:4 and 20:5 increased also to comprise together 10% of the total FA.

In the galactolipids, 16:2, 18:2 and 18:3 were the most abundant FA in all media. Furthermore, 16:4 was found predominantly (11%) in MGDG but only in trace amounts in other lipid classes. Exogenous carbon increased the percentages of 16:0, 18:1 and 20:4 in both galactolipid classes, while nitrogen increased the percentages of 16:3 and 18:3.

In PG, whatever the medium, the summed percentages of 16:0 and *trans*-16:1 were always *ca* 42%. But, according to the nitrogen concentration in the medium, the proportion of each FA was different. With nitrogen in the medium (C − N +), 16:0 was the major FA; without nitrogen in the medium (C − N −, C + N −) *trans*-16:1 was predominant. Although this has not been studied in the case of *Euglena*, these findings suggested, by reference to the two pathways proposed for glycerolipid synthesis in *Arabidopsis* [19], that the chloroplastic FA desaturase A (FAD A) would be partly inhibited by the presence of ammonium in the medium. The contents of 18:2 and 18:3 were essentially equivalent in the three media, suggesting that neither exogenous carbon nor nitrogen influenced their biosynthesis. However, desaturation of 18:0 to 18:1 appeared to be reduced in the presence of ammonium (C − N + medium), as found by Piorreck and Pohl [8]. Lactate (C + N − medium) apparently stimulated this desaturation.

Addition of ammonium phosphate or lactate to the medium at stationary phase

When $(NH_4)_2HPO_4$ (15 mM) was added to the C − N − medium at day 5, the number of cells remained stable as well as the total FA content per cell. Also, the percentage of each FA was not changed significantly.

The addition of $(NH_4)_2HPO_4$ (15 mM) to the C + N − medium at day 5 doubled the cell number and decreased by 50% the FA content per cell. Thus, no synthesis of FA occurred. However, the percentages of

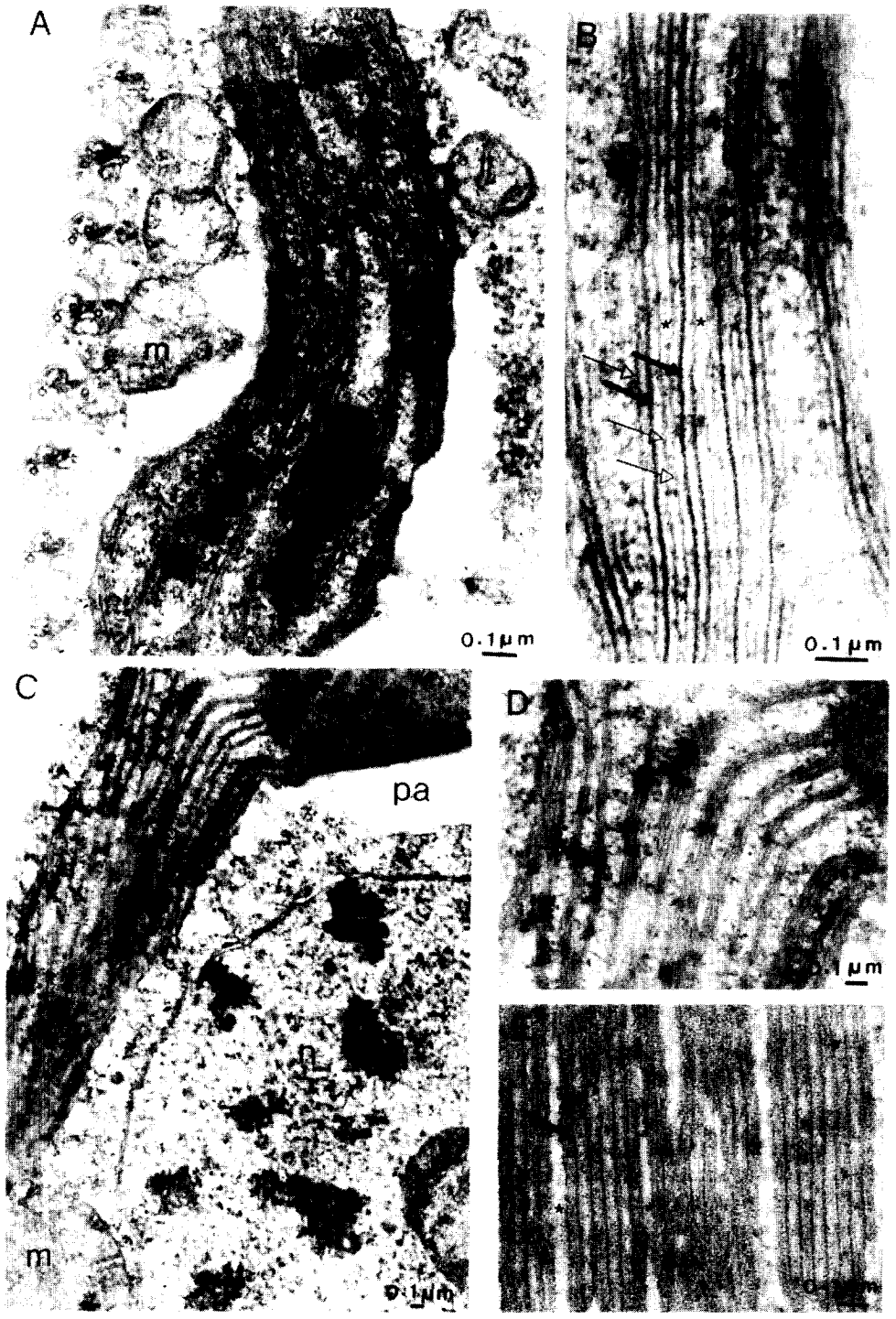

Fig. 3. Electron micrograph of *Euglena gracilis* cells fixed at stationary phase with glutaraldehyde and osmium tetroxide acid and contrasted with uranyl acetate. General view of chloroplast in *Euglena* cell cultivated on C + N − medium (A) and in C − N + medium (C). (B, D, E) Details of long lamellae which cross the chloroplast. Appressed membranes are thicker (➡) than outer membranes (→). These thick membranes were grouped in ones or twos by the maximum in chloroplasts of cells cultivated on C + N − medium (B); or up to sixes or sevens in chloroplasts of cells cultivated on C − N + medium (D, E). li, lipidic droplets; m, mitochondriae; n, nucleus; pa, paramylum; py, pyrenoid; *, stroma.

C_{14} FA, especially 14:0, decreased by five-fold to the benefit of C_{16} PUFA and chiefly of 18:3 that increased by eight-fold. This experiment thus confirmed the stimulating effect of nitrogen on the synthesis of FA characteristic of galactolipids at the expense of short-chain saturated FA.

When lactate (15 mM) was added to C − N − medium at day 5, the number of *Euglena* cells remained constant, whilst the FA content per cell doubled. This proved that there was substantial lipid synthesis from exogenous carbon. All FA contents increased (expressed as pg $cell^{-1}$) but the proportions of saturated and monounsaturated FA increased, specially those of 14:0 and 18:1 which doubled. On the other hand, the proportions of PUFA decreased (essentially 16:4, 18:2 and 18:3 which are mainly present in galactolipids). These FA alterations were correlated with degradation of chloroplasts shown previously [12].

Electron microscopy of chloroplasts

Chloroplasts of *Euglena gracilis* contained long lamellae which crossed this organelle over all its length (Fig. 3). In contrast to higher plant chloroplasts, there were no grana; thylakoids were closed appressed over a long distance. In these regions, the membranes of lamellae were thickened. These dense bands were more or less numerous with respect to culture conditions. On C − N − medium, *Euglena* cells exhibited dense lamellae often grouped in threes. This number does not exceed two in cells cultivated on C + N − medium (Figs 3A and B), while it increased up to six or seven in cells cultivated on C − N + medium (Figs 3C–E). These ultrastructural modifications could be correlated to variations in the MGDG:DGDG ratio; the average number of appressed lamellae increased with an increase in this ratio (*cf.* Table 3). These results are in agreement with those obtained by Brandt and Eichenberger [17] and Gounaris *et al.* [20] with fractions enriched in appressed or non-appressed lamellae, respectively, from *Euglena* or isolated spinach chloroplasts.

CONCLUSION

Our findings clearly indicate that the carbon source of the medium plays a more important role than the nitrogen source with regard to the synthesis of lipids in *Euglena* cells. Nitrogen also promotes the synthesis of galactolipids rich in C_{16} and C_{18} PUFA, whereas lactate promotes the degradation of these chloroplastic lipids.

Our results demonstrate that storage lipids, enriched in saturated C_{14} and C_{16} FA, accumulated when lactate is present in the medium (C + N −). This synthesis is not sensitive to a deficiency in ammonium. In marked contrast, under photoautotrophic conditions (C − N − or C − N + media), storage lipids do not increase and galactolipids, characteristic of chloroplasts, accumulate. The higher the nitrogen content in the medium, the higher the MGDG:DGDG ratio and the greater the number of long appressed thylakoids within the cells.

EXPERIMENTAL

Culture. Euglena gracilis, strain Z, originating from the algotheque of Cambridge (n° 1224-5d) was cultivated photoorganotrophically with 33 mM D, L-lactic acid at 27° under continuous light (3000 lux) without aeration or shaking for 7 days. Mineral salts added to the medium were those of Ref. [21] with the addition of $(NH_4)_2HPO_4$: at 0.95, 1.5 and 3.8 mM; the pH was adjusted to 3. At the stationary phase, lactate and ammonium concns of the three media were distinctly different (Table 1). Thus, three phototrophic conditions were obtained: C + N − medium was characterized by the persistence of lactate, C − N + medium by the persistence of ammonium and C − N − medium by the absence of both lactate and ammonium [12].

Lipid analysis. Euglena cells (at least 30×10^6) collected at exponential and stationary phases were harvested by centrifugation and fixed in boiling H_2O. Lipids were extracted using the method of Ref. [22]. Neutral lipids were extracted from total lipids with Me_2CO (5 ml) and 0.1 ml of MeOH containing 10% $MgCl_2$ [23]. Lipid classes were separated by TLC on silica gel (Kieselgel 60, Merck) with a Lepage solvent [24] and neutral lipids according to Ref. [25]. Lipids were visualized using a spray of primuline (117 mg l^{-1} in Me_2CO–H_2O, 16:1) followed by viewing under UV. Classes were identified by comparison with R_f values of authentic standards.

Aliquots of total lipid or lipid classes were subjected to methanolysis according to Ref. [26] after addition of 19:0 as int. st. The resulting Me esters were extracted with pentane and analysed by GC on a Carbowax fused silica column (50 m long × 0.32 mm diam.). The column was prog. from 80 to 200° during analysis, with injector and detector temps maintained at 250° and 270°, respectively. Peak areas were quantified with integrating software on a PC and FA contents are expressed as pg $cell^{-1}$. The unsaturation index was calculated according to Ref. [27]. Quantitative data represent the means of 3–5 independent expts ± standard deviation.

Electron microscopy. Euglena cells pretreated with glutaraldehyde (1%, v/v) were then harvested by centrifugation (1000 *g*, 10 min) and embedded in Na alginate beads (pH 7.2) according to the method of Ref. [28]. Cells were fixed for 30 min in 0.2% (w/v) OsO_4 in Na cacodylate buffer, pH 7.2. Ultrathin sections were contrasted by uranyl acetate staining.

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