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THE PHOSPHOLIPID FATTY ACIDS OF *PORPHYRIDIUM PURPUREUM* CULTURED IN THE PRESENCE OF TRITON X-100 AND SODIUM DESOXYCHOLATE

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Key Word Index—*Porphyridium purpureum*, Rhodophyceae, cell culture, phospholipids, fatty acids, Triton X-100, sodium desoxycholate, detergents

Abstract—The phospholipid fatty acid composition of *Porphyridium purpureum* grown on a solid medium was studied in the presence of Triton X-100 (TX) and sodium desoxycholate (SDC) The most common fatty acids in PC and PE were palmitic (16 0), stearic (18 0), linoleic (18 2ω6), arachidonic (20 4ω6) and eicosapentaenoic (20 5ω3) acids, 20 4ω6 being very abundant In PG the most common acids were 16 0, *trans*-hexaenoic acid (*tr*16 1ω3), oleic acid (18 1) and 20 4ω6 Both detergents caused an increase in the saturation of PC and, to a lesser extent, of PE The relative amounts of short chain fatty acids increased Both detergents increased the amounts of 16 0 and, correspondingly, decreased the amounts of 20 4ω6 In PG the amounts of both 16 0 and *tr* 16 1ω3 increased and the amounts of 18 0, 18 2ω6 and 20 4ω6 decreased in the presence of detergents The changes were always greatest at the concentrations of 5–10 ppm TX or SDC At 20 ppm the fatty acid compositions, especially with SDC, were very similar to the controls, which suggests a change in the detergent effect between 10–20 ppm The normal PC/PE ratio was 5 6 and the (PC + PE)/PG ratio 39 0 Both detergents caused a marked decrease in these ratios Because the detergent effects are not linear, it seems that even very low detergent concentrations have an important influence on algae in polluted waters

INTRODUCTION

It is known that detergents can inhibit the growth of algae [1, 2] and cause perturbations in their life processes even at concentrations too low to inhibit their growth [3, 4] More information is clearly needed about the reactions of living cells towards detergents, which often contribute to water pollution In an earlier study, the authors investigated the effects of four well-known and much used detergents on the glycolipids of *Porphyridium purpureum*, a halo- and aerophilous unicellular red alga, which is well-suited to this kind of pollution physiology study [5] The present paper is a continuation of that work

The aim of the present work was to find out if detergents influence the fatty acid compositions of the important phospholipids of *P purpureum*, namely PC and PE, which are major constituents of most cellular membranes, and PG, which for the most part is located in the chloroplast membranes together with the glycolipids, whose reactions towards detergents were reported in an earlier paper [5] The ratios PC/PE and (PC + PE)/PG were also assessed to see if detergents could alter them

Very little previous work has been done with the phospholipids of *P purpureum* or of the Rhodophyceae in general [6], although in higher plants and green algae the different phospholipids and their fatty acid compositions are quite well known [7–9] We chose to study PC, PE and PG because they are common and important in *P purpureum* Earlier experience made us choose TX and SDC as suitable detergents for living cell studies, because they do not affect the growth of *P purpureum* very drastically at the concentrations used (5–20 ppm = 8–32 μM for TX and 3–13 μM for SDC) [5] Moreover, these detergents have properties, which make them useful in membrane studies [10, 11] Thus bile salts, such as SDC, bind strongly to, and solubilize, the 'class II swelling amphiphiles' [11], a group to which the phospholipids belong By contrast, they have a very limited capacity to affect aliphatic or aromatic non-polar lipids [10] TX is a classical non-ionic detergent, whose reactions in general appear to be more gentle in membranes than those of the bile salts [11] Both are capable of binding to membrane proteins and lipids, and of traversing the membranes, to gain access to the parts deep inside the cell Because of their non-denaturing character they are very useful when studying the living cell They can affect and change the cellular membranes in various ways, which are reflected in membrane properties and compositions, often in a complicated manner In nature the detergents form one stress factor among many, which the algae have to face To understand detergent pollution better, it is essential to know more about the effects of these compounds on cellular membranes

Abbreviations PC, phosphatidyl choline, PE, phosphatidyl ethanolamine, PG, phosphatidyl glycerol, TX, Triton X-100, SDC, sodium desoxycholate, CMC, critical micellar concentration of the detergent

RESULTS

Normal fatty acid compositions of PC, PE and PG

It has been stated [12] that PC and PE usually have a very similar fatty acid composition in plants, a fact which

we confirmed for *P purpureum* The dominant fatty acids in the PC and PE of this alga were arachidonic (20 4ω6) and palmitic (16 0) acids, which together accounted for over 50% of the total fatty acids in these phospholipids (Tables 1, 2, 4 and 5) Other prominent acids in PC and PE were 18 0, 18 2ω6 and some members of the C_{20} series, although eicosapentaenoic acid (20 5ω3) was not as important as in the glycolipids of *P purpureum* [5] γ-Linolenic acid (18 3ω6) was usually present in small amounts, but only putative traces were found of α-linolenic acid (18 3ω3) Some unusual fatty acids were present in both PC and PE, although in such small amounts that definite identification was not always possible 14 3 (not separable from 16 1 [5]), 19 0, 22 1, 22 5ω3 and 23 0 (its existence in *P purpureum* was confirmed by MS data) Polyunsaturated C_{16} acids were very rare The great differences between the amounts of the 'major' and 'minor' acids seem to be typical of *P purpureum* cultured under these conditions [5]

The PG of *P purpureum* differed from the other two phospholipids studied in containing a large amount of *trans*-hexaenoic acid (*tr*16 1ω3) (Tables 3 and 6), which is in most plants a typical fatty acid in PG, but is rarely found elsewhere, in contrast to PC and PE, PG is situated in the chloroplast [8] Otherwise the PG fatty acid composition observed was not very different from that of PC and PE, except that it contained a proportionally smaller amount of 20 4ω6 It also had a slightly higher percentage of short chain (C_{14-15}) fatty acids Both types of linolenic acid were present in small amounts in PG

Effects of TX and SDC on the fatty acid compositions of PC and PE (Tables 1, 2, 4 and 5)

A general tendency seemed to be that the greatest differences from controls were to be seen at the concentrations of 5–10 ppm This phenomenon was observed earlier for the *P purpureum* glycolipids [5] The amount of 16 0 increased markedly in the presence of 5–10 ppm TX, and this pattern was followed also by 18 0 and 18 1, whose amounts, however, continued to rise with increasing detergent concentration (Tables 1 and 2) Both detergents increased the amounts of the C_{14-15} acids to some extent The amounts of 20 4ω6 always showed a corresponding drop in the presence of TX, although this was more marked in PC The levels of 20 5ω3 seemed to decrease somewhat in both phospholipids in the presence of TX No great changes were observed in the amounts of 18 2ω6

The effects of SDC resembled those of TX SDC caused a great increase in the amount of 16 0 at the concentration of 5–10 ppm in PC, which corresponded with a simultaneous decrease in the amount of 20 4ω6 These changes were not as clear in PE (Tables 4 and 5) In PC the levels of 18 0 and 18 1 followed the pattern of 16 0, while 18 2ω6 displayed the reverse behaviour having a minimum at 5–10 ppm SDC At 20 ppm SDC, the fatty acid compositions were always very close to the controls SDC had little effect on the amounts of 20 5ω3 in PC or PE

Effects of TX and SDC of the fatty acid composition of PG (Tables 3 and 6)

Contrary to expectations, the amounts of both 16 0 and *tr*16 1ω3 were found to increase at the detergent concentrations of 5–10 ppm Usually these acids have been observed to be linked with each other so that when the amount of one increases, the other decreases [8] At the concentration of 20 ppm TX or SDC the amounts of both were returned to the control levels again These changes seemed mostly to occur at the expense of 18 0, 18 2ω6 and 20 4ω6 A temporary high level in the amount of 20 2ω9 was observed at the concentration of 10 ppm SDC

Effects of TX and SDC on the PC/PE and (PC + PE)/PG ratios (Table 7)

As expected, PC was quantitatively the most abundant of the three studied phospholipids in *P purpureum* The amount of PE was also notably high, but the amount of PG was according to the quantitative measurements (Table 7) very small in the controls compared with the others The detergents caused a drastic decrease in both ratios, our experiments indicated that this was mainly as a result of an increase in the amounts of PE and PG, which is seen here as a decrease of both ratios By contrast, the amounts of PC seemed to be quite stable in the presence of TX or SDC TX and SDC were earlier shown to be able also to influence the glycolipid MGDG/DGDG ratio [5]

DISCUSSION

According to the findings of Nichols and Appleby [6], *P purpureum* contains only small amounts of phospholipids other than PC and PG PC is usually the major lipid for algal plasma membranes [13] We found, however, that under the growth conditions used, the PE levels were quite high (Table 7), whereas the amounts of phosphatidyl serine and phosphatidyl inositol were negligible The high PE level is, in our experiments, probably a result of the growth temperature used (25°) Indeed it has been stated that the amount of PE increases with temperature in *Cyanidium*, a thermophilic alga [14], whereas the PC level is not greatly influenced by temperature As to the PG level of the cells, it is more a function of the conditions In etiolated plants [12, 15] and non-photosynthetic tissues [16] its amount is very small, in keeping with the fact that it is almost entirely located in the chloroplasts, although minor amounts have been found in other organelles, e g mitochondria [8] The light conditions used here [5] seemed to be favourable for PG synthesis

The detergents caused dramatic changes in the relative amounts of the phospholipids studied (Table 7) The quantitative experiments indicated that the PC content of the cells was quite stable, and that the detergents induced a marked increase in the amounts of both PE and PG (Table 7) This is in accord with earlier studies [12, 14–16], which show that PE and PG are more susceptible to changes in environmental conditions Detergents are known to decrease photosynthesis [5] and perhaps this is reflected in the changes observed in the PG amounts

P purpureum synthesizes large quantities of 20 4ω6 using 18 3ω6 as an intermediate [6] Accordingly we found that 20 4ω6 was the most common fatty acid in PC and PE, followed by 16 0 (Tables 1, 2, 4 and 5) Other acids of the C_{20} series were also well represented, although a fairly high growth temperature favours the formation of 16 0 [14] The levels of unsaturation of PC and PE are also very dependent on their localization in the cell chloroplast PC is more unsaturated than other PCs [15],

Table 1 Relative amounts (%) of fatty acids in *P purpureum* PC in the presence of Triton X-100 (TX)†

		[TX] in growth medium (ppm)			
Acid	Control	5	10	15	20
14 0	0 5	2 2**	1 4*	0 8	1 4
14 1	tr	tr	0 5	0 4	tr
14 3+16 1	1 4	1 9	4 8*	2 8**	3 3*
15 0	tr	1 3	1 4	0 8	0 8
16 0	22 3	59 6***	42 1**	22 9	21 8
*tr*16 1ω3	—	—	—	—	0 4
17 0	0 3	0 8***	1 0	0 8	0 9
18 0	5 6	6 5	6 0	9 1	19 0***
18 1	1 8	2 5	4 1*	5 5**	10 3***
18 2ω6	12 0	3 8***	10 5	6 9***	8 0**
18 3ω6	1 5	0 4	0 9	0 8	0 9
19 0	2 1	tr	—	0 5	—
20 1	tr	—	tr	0 8	—
20 2ω9	0 9	2 3	1 2	1 1	2 0
20 3ω6	1 8	tr	1 2	2 8	2 8
20 4ω6	40 8	11 3**	14 1**	34 1	11 3***
20 5ω3	5 4	3 5	1 8**	3 8	3 3
22 1	0 3	0 8	1 4**	0 7	0 4
22 5ω3	—	—	tr	—	—
23 0	tr	—	0 6	0 6	0 4
24 0	0 4	tr	0 6	1 0	1 2
24 1	tr	0 5	2 0	tr	0 7

†Average percentages based on 8–10 independent samples, expressed as % of total fatty acid All results were statistically tested with Student's *t* test * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$ tr, < 0 2%

Table 2 Relative amounts (%) of fatty acids in *P purpureum* PE in the presence of Triton X-100 (TX)†

		[TX] in growth medium (ppm)			
Acid	Control	5	10	15	20
14 0	0 3	1 5*	1 1**	2 0***	1 6***
14 1	—	tr	tr	0 8	tr
14 3+16 1	0 9	2 4***	2 3**	4 2***	3 0***
15 0	0 2	1 2***	0 7**	1 4***	0 7***
16 0	22 9	25 0	32 3	35 8*	18 4*
*tr*16 1ω3	tr	—	—	—	0 5
17 0	0 2	0 5	0 4	0 6*	0 5
18 0	10 7	6 0	10 1	12 4	18 0**
18 1	2 5	2 7	2 3	5 3***	7 3***
18 2ω6	4 3	1 8**	3 8	3 8	3 1
18 3ω6	0 3	0 3	0 5	0 7	0 3
19 0	2 8	0 2	tr	1 0	0 6
20 1	0 3	—	—	0 6	1 9***
20 2ω9	4 1	1 8	2 5	5 6	2 2*
20 3ω6	2 3	1 7	0 9*	0 8**	2 4
20 4ω6	34 2	40 5	32 0	7 1**	27 2
20 5ω3	9 6	7 0	4 7*	9 2	4 1**
22 1	0 3	0 9	1 0	0 6	0 5
22 5ω3	—	1 5	1 6	—	—
23 0	tr	0 7	tr	1 3	0 6
24 0	0 6	0 6	0 8	1 3	1 1
24 1	tr	1 0	0 6	tr	0 4

†See Table 1 for explanations of symbols used

Table 3. Relative amounts (%) of fatty acids in *P. purpureum* PG in the presence of Triton X-100 (TX)†

		[TX] in growth medium (ppm)			
Acid	Control	5	10	15	20
14:0	1.0	2.2	1.5	2.3*	2.3
14:1	0.3	0.3	0.7	1.0	0.4
14:3 + 16:1	1.4	1.9	0.9	2.6***	5.0***
15:0	0.7	1.6*	1.6**	1.4*	1.4*
16:0	26.9	41.2*	40.3*	31.3	21.8
*tr*16:1ω3	11.8	22.3**	23.4***	9.9	8.8
17:0	0.8	1.1	0.9	0.8	0.7
18:0	14.9	7.4*	7.1*	9.4*	18.7
18:1	5.9	4.6	3.4*	5.2	12.2***
18:2ω6	2.8	0.4**	0.8**	2.8	1.5
18:3ω6	1.3	0.4	0.7	tr	0.3
19:0	—	—	0.3	0.4	—
20:1	tr	—	—	0.3	tr
20:2ω9	1.0	2.0	0.8	2.3*	0.9
20:3ω6	0.9	—	0.3	1.6	3.1**
20:4ω6	10.9	4.6*	1.3***	7.3	3.6***
20:5ω3	5.4	1.7*	1.0**	4.3	2.9*
22:1	0.9	0.8	1.9	1.0	0.9
22:5ω3	—	1.9	0.7	4.4	—
23:0	0.7	—	0.9	0.2	1.0
24:0	0.8	0.5	2.6	2.7**	1.7
24:1	tr	1.7	1.1	1.6	1.0

†See Table 1 for explanations of symbols used.

Table 4. Relative amounts (%) of fatty acids in *P. purpureum* PC in the presence of sodium desoxycholate (SDC)†

		[SDC] in growth medium (ppm)			
Acid	Control	5	10	15	20
14:0	0.5	0.9	0.9	tr	0.9
14:1	tr	tr	tr	tr	tr
14:3 + 16:1	1.4	1.9	3.4*	0.9	1.9
15:0	tr	0.9**	0.7*	0.3	0.5
16:0	22.3	41.9**	44.1***	26.9	23.5
*tr*16:1ω3	—	—	—	—	tr
17:0	0.3	0.8***	0.5	0.6	0.4
18:0	5.6	7.8***	15.5**	10.5**	6.1
18:1	1.8	3.5**	3.8*	2.4	1.8
18:2ω6	12.0	4.9***	4.1***	14.2	11.6
18:3ω6	1.5	0.4	0.4	3.0	1.3
19:0	2.1	tr	0.4	0.6	3.1
20:1	tr	—	—	0.5	—
20:2ω9	0.9	1.3	3.2*	1.6	1.0
20:3ω6	1.8	1.9	0.7	1.5	2.7
20:4ω6	40.8	21.3*	6.8***	21.0*	35.8
20:5ω3	5.4	4.4	5.1	5.7	5.5
22:1	0.3	0.5	1.6	1.4	0.6
22:5ω3	—	4.6	—	—	—
23:0	tr	—	—	2.4	0.5
24:0	0.4	tr	3.9	2.3	0.9
24:1	tr	0.7	2.6	tr	tr

†See Table 1 for explanations of symbols used.

Table 5. Relative amounts (%) of fatty acids in *P. purpureum* PE in the presence of sodium desoxycholate (SDC)†

		[SDC] in growth medium (ppm)			
Acid	Control	5	10	15	20
14:0	0.3	0.3	2.7*	0.3	0.6
14:1	—	tr	0.5	tr	tr
14:3 + 16:1	0.9	1.0	5.9***	0.6	1.5
15:0	0.2	0.5	2.1***	0.2	0.3
16:0	22.9	18.3	38.3*	12.0	27.1
*tr*16:1ω3	tr	—	—	—	—
17:0	0.2	0.4	0.8**	tr	0.3
18:0	10.7	4.7*	9.3	3.7**	11.1
18:1	2.5	2.3	5.3**	4.0	2.4
18:2ω6	4.3	1.9**	1.7**	5.6	4.2
18:3ω6	0.3	0.2	0.4	0.8	0.4
19:0	2.8	tr	0.4	tr	2.8
20:1	0.3	—	tr	0.5	0.6
20:2ω9	4.1	1.1*	2.3	1.8	3.6
20:3ω6	2.3	2.8	0.8**	3.0	2.3
20:4ω6	34.2	53.1	14.5*	41.7	30.8
20:5ω3	9.6	9.0	4.5*	9.2	9.3
22:1	0.3	0.5	1.9	2.4	0.5
22:5ω3	—	1.2	tr	—	—
23:0	tr	—	0.5	7.5**	tr
24:0	0.6	tr	2.2	1.2	0.6
24:1	tr	0.8	1.7	1.5	tr

†See Table 1 for explanations of symbols used.

Table 6. Relative amounts (%) of fatty acids in *P. purpureum* PG in the presence of sodium desoxycholate (SDC)†

		[SDC] in growth medium (ppm)			
Acid	Control	5	10	15	20
14:0	1.0	0.9	1.4	0.9	1.3
14:1	0.3	0.3	tr	0.2	tr
14:3 + 16:1	1.4	0.7	0.6	1.1	1.2
15:0	0.7	1.2	1.2	0.8	0.7
16:0	26.9	36.1*	33.7	26.2	25.3
*tr*16:1ω3	11.8	22.6***	12.5	10.3	10.7
17:0	0.8	0.9	1.3	0.6	0.6
18:0	14.9	8.5**	11.3	3.9***	17.8
18:1	5.9	4.0	2.0**	5.0	5.5
18:2ω6	2.8	0.8***	0.5***	1.7	1.8
18:3ω6	1.3	0.4	0.8	0.2	0.6
19:0	—	—	—	0.5	5.5
20:1	tr	tr	—	1.2	tr
20:2ω9	1.0	2.3	15.9**	0.8	2.1
20:3ω6	0.9	1.3	1.6	2.9*	1.1
20:4ω6	10.9	6.4	4.2*	9.7	12.6
20:5ω3	5.4	5.6	4.5	7.1	4.5
22:1	0.9	1.6	1.4	tr	1.0
22:5ω3	—	tr	—	—	—
23:0	0.7	0.4	—	8.9	0.9
24:0	0.8	5.3**	3.4**	5.3**	1.9
24:1	tr	3.3	1.7	1.1	tr

†See Table 1 for explanations of symbols used.

Table 7. Amounts of PC, PE and PG, and PC/PE and (PC + PE)/PG ratios in *P. purpureum* cultured in the presence of Triton X-100 (TX) and sodium desoxycholate (SDC)†

Detergent		Amount (μgP/g dry wt.)			Ratios	
Name	(ppm)	PC	PE	PG	PC/PE	(PC/PE)/PG
—	0	20.0	3.6	0.6	5.6	39.0
TX	5	26.0	3.0	5.4***	8.7	5.4***
	10	26.9	17.9	25.1*	1.5***	1.8***
	15	18.5	12.3	8.3	1.5***	3.7**
	20	33.0	34.8*	26.6*	0.9***	2.5***
SDC	5	20.3	27.7**	19.7*	0.7***	2.4***
	10	32.0	14.4	34.3*	2.2**	1.4***
	15	21.2	14.1	9.9	1.5***	3.6***
	20	12.8	6.8	3.6	1.9*	5.4***

† Average of five independent determinations. See table 1 for explanation of symbols used.

and PC and PE are more unsaturated in the inner mitochondrial membranes than in the outer [7].

The major fatty acids in *P. purpureum* PG were 16:0, 18:0, *tr*16:1ω3 and 20:4ω6, in this order (Tables 3 and 6). In green algae, PG also tends to be rich in 16:0, although 18:3ω3 has a central role [15]. The amount of *tr*16:1ω3, an acid characteristic for PG, is very much dependent on light: in etioplasts and in darkness it is usually present only in traces [8, 16]. The acids 16:0 and *tr* 16:1 ω3 are linked with each other because it seems that the latter is synthesized from 16:0 in light and is converted back in dark [15, 17].

The changes observed with detergents in the fatty acid compositions of the studied phospholipids resemble the changes noticed earlier in the glycolipid fatty acid compositions of *P. purpureum* [5]. The greatest differences from controls were found at the concentrations of 5–10 ppm of both TX and SDC. Usually the changes are expressed as increases in the degree of saturation, especially in PC. The same changes are seen in PE, but are not as marked. In PG the amount of *tr*16:1ω3, somewhat surprisingly, increased along with the 16:0 amount.

Even much lower concentrations of TX than 5–10 ppm have been found to cause instability of the mitochondrial phospholipid bilayers and reduction of their direct current resistance, thus causing an increase in permeability, when a detergent–cation–lipid complex is formed [18]. Our results also indicate infringement upon the lipid biosynthesis mechanisms [11]. It has been found that TX can cause complete inhibition of oleyl-CoA-desaturase even at a concentration of 5 ppm [19]. By binding to the membranes and gradually extracting the lipid part around the enzyme submerged in the membrane, the detergent changes the natural environment of the enzymes, and harmful effects follow. These phenomena have been observed with both TX and SDC [7, 19, 20]. By using concentrations lower than their CMC, the detergents infringe also upon the lipid exchange systems between membranes, which are mediated by the cytoplasmic phospholipid exchange proteins or PLEP's [9]. Sub-CMC-concentrations of detergents do not cause delipidation of the PLEP and then the hydrolytic actions of various phospholipases are greatly enhanced [21, 22], which results in changes in the proportional amounts of different phospholipids.

TX and SDC appear to interact mainly with proteins which are bound to the membrane lipids by hydrophobic interactions; usually, low concentrations of these detergents do not cause major changes in the proteins or their activity [11]. However, it has been stated that the bile salts especially can cause lysis far below the CMC [21], and the monomer form is well able to penetrate membranes causing localized disorders [11]. In the conditions used in our experiments, it can be assumed that the detergents occur both as monomers and as micelles, because of their low CMC and the high electrolyte concentration of the medium [5, 10]. The CMC of SDC is at a minimum level between 20° and 30°, and the CMC of TX is much lower, which is usual for non-ionics [10].

Our results indicate a change in the detergent behaviour above the concentration of 10 ppm (see Tables), which most probably is a result of some change in e.g. the monomer/micelle ratio of the detergent. It is difficult to know whether the phenomena observed are determined by the membrane rather than the detergent [11]. However, we have confirmed the potential harmfulness of very low detergent concentrations, which is in accord with our earlier results [5], and is an important fact concerning the pollution of natural waters.

EXPERIMENTAL

Plant material. The unicellular, halophilous red alga *Porphyridium purpureum* (Bory) Ross (syn. *P. cruentum* (Ag.) Naeg.) strain CCAP 1380/1a (Cambridge, England) was used as a pure culture.

Culture. The alga was cultured on a solid 1% agar nutrient medium after ref. [5] containing the following macro nutrients (ppm): NaCl 27000, $MgSO_4$ 3200, $MgCl_2$ 2600, $CaCl_2$ 1300, KNO_3 1000, KH_2PO_4 70 and $NaHCO_3$ 40. The medium was buffered with 1 M Tris–HCl, pH 7.6 (20 ml/l), Fe was added as an 8.3 mg/ml Fe^{3+} Na-EDTA solution (1 ml/l). The micro nutrient soln used (1 ml/l) contained (in ppm): $ZnCl_2$ 40, H_3BO_3 600, $CoCl_2$ 8.2, $CuCl_2$ 31.6, $MnCl_2$ 254 and $(NH_4)_6MoO_{24}$ 370.

Growth and harvest. The organism was grown for 1 month in Petri dishes (7 cm diam.) at 25° under Airam 40W-35 white

fluorescent tubes giving on average 100 $\mu E/m^2/sec$ (400–700 nm) 18 hr/day. At the end of the period the tough algal layer was removed quantitatively from the agar surface and lyophilized. Every sample consisted of the combined harvested algae of four Petri dishes.

Detergents. Two 'mild type' detergents were used: (1) Triton X-100® (TX, iso-octylphenoxypolyethoxyethanol, non-ionic, CMC 0.24 mM in H_2O) and (2) sodium desoxycholate (SDC, anionic, CMC 4–6 in H_2O). For the characteristics of these compounds see refs [10, 11, 18, 23]. Both detergents were of analytical grade and they were added to the slightly cooled autoclaved medium using 0.22 μm sterile Millipore® filters to avoid their possible decomposition during sterilization.

Lipid analysis. The lipids were extracted with $CHCl_3$–MeOH (2:1) and fractioned in a silicic acid column [5]. The phospholipids were separated using TLC plates of silica gel $60F_{254}$ with concentrating zone. The plates were developed with $CHCl_3$–MeOH–7 M NH_3 (115:45:7.5) [24], and the phospholipids located as in refs [5, 25]. Extraction from the plates was done using $CHCl_3$–MeOH (2:1). The identification of the phospholipids was performed using specific stains [25, 26], commercial standards, and 2D-TLC [24]. The PC/PE and (PC + PE)/PG ratios were determined spectrophotometrically [27].

The PC, PE and PG fatty acid compositions were determined by GC/MS using methyl esters as derivatives [28]. The GC/MS analyses were performed using methods and instruments previously described [5]. ECL values were used according to the principle of Jamieson [29] to determine the double bond positions in the polyunsaturated fatty acid methyl esters.

Statistics. The results are average percentages based on 6–8 independent samples, expressed as % of the total fatty acids. The statistical significance of all results was tested with the Student's *t* test: * = $p \leqslant 0.05$, ** = $p \leqslant 0.01$, *** = $p \leqslant 0.001$.

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REFERENCES

1. Ukeles, R. (1965) *J. Phycol.* **1**, 102.
2. Nyberg, H. (1976) *Ann. Bot. Fennici* **13**, 65.
3. Labus, B. C. and Kohler, A. (1981) *Limnologica* **13**, 373.
4. Nyberg, H. (1981) *Ann. Bot. Fennici* **18**, 37.
5. Nyberg, H. and Koskimies-Soininen, K. (1984) *Phytochemistry* **23**, 751.
6. Nichols, B. W. and Appleby, R. S. (1969) *Phytochemistry* **8**, 1907.
7. Mazliak, P. (1977) in *Lipids and Lipid Polymers in Higher Plants* (Tevini, M. and Lichtenthaler, H. K., eds). Springer, Berlin.
8. Harwood, J. L. (1980) in *The Biochemistry of Plants* (Stumpf, P. K., ed.) Vol. 4, Academic Press, New York.
9. Mazliak, P. and Kader, J. C. (1980) in *The Biochemistry of Plants* (Stumpf, P. K., ed.) Vol. 4. Academic Press, New York.
10. Carey, M. C. and Small, D. M. (1972) *Am. J. Med.* **49**, 590.
11. Helenius, A. and Simons, K. (1975) *Biochim. Biophys. Acta* **415**, 29.
12. Mudd, J. B. (1980) in *The Biochemistry of Plants* (Stumpf, P. K., ed.) Vol. 4, Academic Press, New York.
13. Bailey, D. S. and Northcote, D. H. (1976) *Biochem. J.* **156**, 295.
14. Kleinschmidt, M. G. and McMahon, V. A. (1970) *Plant Physiol.* **46**, 290.
15. Rosenberg, A. (1973) in *Lipids and Biomembranes of Eukaryotic Microorganisms* (Erwin, J. A., ed.). Academic Press, New York.
16. Fishwick, M. J. and Wright, A. J. (1980) *Phytochemistry* **19**, 55.
17. Selldén, G. and Selstam, E. (1976) *Physiol. Plant.* **37**, 35.
18. van Zutphen, H., Merola, A. J., Brierley, G. P. and Cornwell, D. G. (1972) *Arch. Biochem. Biophys.* **152**, 755.
19. Mazliak, P. (1975) *C. R. Acad. Sci.* **281**, 1471.
20. Mazliak, P. and Decotte, A. M. (1976) *J. Exp. Botany* **27**, 769.
21. Roelòfsen, B., Zwaal, R. F. A., Comfurius, P., Woodward, C. B. and van Deenen, L. L. M. (1971) *Biochim. Biophys. Acta* **241**, 925.
22. van Deenen, L. L. M., Demel, R. A., Geurts van Kessel, W. S. M., Kamp, H. H., Roelofsen, B., Verkleij, A. J., Wirtz, K. W. A. and Zwaal, R. F. A. (1976) in *The Structural Basis of Membrane Function* (Hatefi, Y. and Djavanie-Ohaniance, L., eds). Academic Press, New York.
23. Bjerrum, O. J., Bhakdi, S. and Rieneck, K. (1980) *J. Biochem. Biophys. Methods* **3**, 355.
24. Abramson, D. and Blecher, M. (1964) *J. Lipid Res.* **5**, 628.
25. Kates, M. (1972) *Techniques of Lipidology*, pp. 436–437. North-Holland, Amsterdam.
26. Dittmer, J. C. and Lester, R. L. (1964) *J. Lipid Res.* **5**, 126.
27. Rössel, T. and Kiesslich, H. (1967) *Fresenius Z. Anal. Chem.* **229**, 96.
28. Klopfenstein, W. E. (1971) *J. Lipid Res.* **12**, 773.
29. Jamieson, G. R. (1975) *J. Chromatogr. Sci.* **13**, 491.