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THE INFLUENCE OF IONIC DETERGENTS ON THE PHOSPHOLIPID FATTY ACID COMPOSITIONS OF *PORPHYRIDIUM PURPUREUM*

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Key Word Index—*Porphyridium purpureum*; Rhodophyceae; cell culture; phospholipids; fatty acids; detergents; sodium dodecyl sulphate; cetyl trimethylammonium bromide.

Abstract—The phospholipid fatty acid composition of *Porphyridium purpureum* on a solid medium was studied in the presence of sodium dodecyl sulphate (SDS) and cetyl trimethylammonium bromide (CTAB). The most common fatty acids in phosphatidyl choline (PC) and phosphatidyl ethanolamine (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 phosphatidyl glycerol (PG) the most common acids were 16:0, *trans*-hexadecenoic acid (*tr* 16:1ω13), oleic acid (18:1) and 20:4ω6. Both detergents increased the saturation grade of PC and PE by decreasing the relative amount of the polyunsaturated acids, especially 20:4ω6. A corresponding increase in the amounts of saturated acids was observed in PC and PE. The changes in PG fatty acid composition were not very significant: a slight increase was observed in the amounts of 16:0 and *tr* 16:1ω13, with a corresponding decrease in the amounts of 20:4ω6 and 20:5ω3. Both detergents decreased the PC/PE and the (PC + PE)/PG ratios very markedly, most probably as a result of increases in the amounts of PE and PG. In the presence of CTAB the cells seemed to contain much more phospholipids than in the presence of SDS, perhaps as a result of the mucilage-precipitating effect of CTAB. The significance of the findings is discussed.

INTRODUCTION

SDS and CTAB belong to the denaturing ionic detergents [1]. Extensive work has not been made concerning their harmful effects on living cells, in spite of the fact that the common use of SDS in membrane studies and electrophoresis has up to now been more or less based on empirical reasons [2]. CTAB is a typical representative of the quaternary ammonium compounds, an important cationic detergent group. It serves as disinfectant, emulsifier, hydrotropic compound and detergent in some special washing agents; it is also used in cosmetics [3]. Both SDS and CTAB have been used in biochemistry, especially the former [1, 4, 5], but some data also exist on CTAB [5–7]. Algal cell growth is strongly inhibited by both [8, 9].

An earlier paper [9] presented the effects of SDS, CTAB, and two non-denaturing type detergents, TX and SDC, on the glycolipid fatty acid composition of *P. purpureum*, a unicellular, halophytic red alga, and a second piece of work in this series [10] was concerned with the phospholipid fatty acid composition of this species cultured in the presence of TX and SDC. The present study, which is a continuation of the above mentioned papers, presents the effects of SDS and CTAB on the phospholipid fatty acid compositions of *P. purpureum*. Three important phospholipids were investigated, namely PC and PE, which are major components of most cellular membranes, and PG, which is for the most part situated in the chloroplast membranes and differs from the other two with regard to its fatty acid composition [10]. The non-denaturing detergents TX and SDC were earlier [10] found to have a very marked effect on the PC/PE and (PC + PE)/PG ratios of *P. purpureum*, and therefore these were also assessed in the present study.

SDS and CTAB kill the cells of *P. purpureum* at concentrations over 5 ppm (SDS 17 μM, CTAB 14 μM) in the solid agar growth medium used in these studies [9]. It is well known that in such low concentrations the detergent monomer form is the effective form, which binds to membranes (i.e. to their lipids and proteins [1]), until the detergent concentration reaches the critical micellar concentration (CMC), a fairly narrow concentration range dependent on many physical and chemical factors, where micelles start to form. The nutrient medium used contained a large amount of NaCl (2.7%) because of the halophytic nature of *P. purpureum*, and also other salts; this is found to decrease the CMC strongly. It drops (e.g. in SDS from 8.1 mM to 0.5 mM) if 0.5 M NaCl is present in the solution, and the CMC of CTAB approaches zero in ionic solutions [11]. It can be assumed that both monomers and micelles exist in the conditions used in these studies.

Both SDS and CTAB seem to be biodegradable [12, 13] and thus their concentrations would decrease fairly rapidly in natural waters. Bacteria in particular play a central role in biodegradation; however, some degradation products can be poisonous. Many algae are known to have an ability to degrade detergents in a fairly short time [14, 15]. The biodegradation is very much subject to environmental factors, and even very low concentrations of SDS and CTAB have been observed to have harmful effects on algae [8, 9].

RESULTS

The normal fatty acid compositions of *P. purpureum* PC, PE and PG are presented in Tables 1–3. The charac-

Table 1. Percentage amounts of fatty acids in *P. purpureum* PC in the presence of sodium dodecyl sulfate (SDS) and cetyl trimethylammonium bromide (CTAB)†

		Concentration of SDS and CTAB (ppm)			
		SDS		CTAB	
Acid	Control	2.5	5.0	2.5	5.0
12:0	tr	tr	tr	0.3	0.3
14:0	0.5	3.6*	1.4	2.1	1.5
14:1	tr	1.2	0.5	0.3	0.7
14:3+16:1	1.4	10.5*	3.5	5.2	2.3
15:0	tr	2.7	1.3	1.5	2.0
16:0	22.3	31.3*	28.9	29.2*	42.0*
tr 16.1ω 13	—	tr	tr	—	1.0
16:2ω3	—	1.1	1.0	0.3	0.5
16:2ω6	—	tr	tr	0.6	0.4
16:3ω6	tr	tr	0.8	0.3	tr
17:0	0.3	1.1*	0.6	0.6	1.2*
18:0	5.6	7.3	9.2	15.3*	13.9*
18:1	1.8	13.9*	4.9	5.7*	2.7
18:2ω6	12.0	3.9**	3.4**	5.1**	1.8**
18:3ω6	1.5	tr	0.5*	—	0.4
20:1	tr	0.6	tr	1.6	tr
20:2ω9	0.9	1.8	1.8	0.6	1.9
20:3ω6	1.8	1.7	1.0	0.5	tr
20:4ω6	40.8	4.2***	19.2	7.0***	6.9**
20:5ω3	5.4	0.6*	6.5	2.7	2.9
22:1	0.3	tr	2.0	1.2	2.1
23:0	tr	tr	1.1	1.1	0.3
24:0	0.4	0.6	5.5	13.0**	7.4*
24:1	tr	tr	1.6	0.6	2.4

†Average percentages based on 6–8 independent samples, expressed as % of total fatty acid. tr: % < 0.2. All results were statistically tested with Student's *t* test: * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

teristic features of these phospholipids in these culture conditions are more comprehensively treated in an earlier paper [10]. The fatty acid compositions of PC and PE are quite similar to each other, which is the usual situation in plants [16]. 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*-hexadecenoic acid (*tr* 16:1ω13), oleic acid (18:1) and 20:4ω6, thus differing from the other two.

Effects of SDS and CTAB on the fatty acid compositions of PC and PE (Tables 1 and 2)

The overall tendency seemed to be an increase in the saturation grade in the fatty acid compositions of both phospholipids: this was especially marked in PE, although the results were statistically more significant in PC. Both detergents caused a decrease in the amounts of 18:2ω6, 20:3ω6 and especially 20:4ω6, where the change was really prominent. CTAB also caused a decrease in the amounts of 20:2ω9 and 20:5ω3. Correspondingly, the amounts of especially 16:0, 18:0, 18:1, and lignoceric (24:0) and nervonic acid (24:1) increased in both PC and PE. Small increases could also be observed in the amounts of short chain (C_{12}–C_{15}) fatty acids. SDS and CTAB also seemed to facilitate the occurrence of some unsaturated C_{16} acids, which usually were not present in the controls. This result was not observed in the presence of TX or SDC [10]. Otherwise the effects of SDS and CTAB resembled the earlier results obtained with the non-denaturing detergents, although SDS and CTAB caused marked effects already at much lower concentrations, which is in accord with their denaturing nature and growth inhibition effects [9].

Effects of SDS and CTAB on the fatty acid composition of PG (Table 3)

The changes in the PG fatty acid composition were not very significant compared with those in PC and PE. However, the changes were in the same direction; the relative amounts of especially 20:4ω6 and 20:5ω3 decreased, and the amounts of the short chain (C_{12}–C_{15}) acids, 16:0, 24:0 and 24:1 increased in the presence of SDS and CTAB. This was also true of *tr* 16:1ω13, although it is an unsaturated acid and more often a decrease in the amount of this acid has been observed if the 16:0 amount increases [17]. However, *tr* 16:1ω13 behaved similarly in the presence of TX and SDC [10]. The effects of CTAB seemed to be a little more clear than those of SDS. In the

Table 2. Percentage amounts of fatty acids in *P. purpureum* PE in the presence of sodium dodecyl sulfate (SDS) and cetyl trimethylammonium bromide (CTAB)†

		Concentration of SDS and CTAB (ppm)			
		SDS		CTAB	
Acid	Control	2.5	5.0	2.5	5.0
12:0	tr	0.6	—	0.8	0.4
14:0	0.3	2.0	0.9	1.6	2.1
14:1	—	tr	0.5	0.3	0.8
14:3+16:1	0.9	7.4	0.8	4.7	3.0
15:0	0.2	3.6	1.0	1.0	2.0
16:0	22.9	25.4	44.4*	17.8	48.8
tr 16.1ω 13	tr	—	2.3	—	—
16:2ω3	0.2	1.2	0.7	0.8	0.6
16:2ω6	—	tr	0.4	0.7	0.8
16:3ω6	—	0.3	tr	tr	—
17:0	0.2	0.5	0.6	0.5	0.5
18:0	10.7	8.7	10.7	13.4	14.5
18:1	2.5	9.5	4.1	6.4	3.5
18:2ω6	4.3	3.1	1.7	4.3	0.4*
18:3ω6	0.3	tr	0.8	0.5	0.2
20:1	0.3	tr	—	2.5	—
20:2ω9	4.1	4.0	3.5	0.7	1.7
20:3ω6	2.3	1.6	0.7	tr	tr
20:4ω6	34.2	7.4	6.5	11.4	1.4*
20:5ω3	9.6	3.0	8.2	5.4	1.8*
22:1	0.3	tr	1.3	1.7	1.0
23:0	tr	4.7	1.4	1.2	1.0
24:0	0.6	5.4	3.0	11.4**	7.6
24:1	tr	2.1	1.2	1.9	2.1

†See Table 1 for explanations for symbols used.

presence of SDS the amounts of 18:0, 18:1 and 20:2ω9 increased to some extent, while the reverse was observed in the presence of CTAB. PG seemed to be more resistant towards detergent-induced changes than PC or PE.

Effects of SDS and CTAB on the PC/PE and (PC + PE)/PG ratios (Table 4)

Both detergents caused a clear decrease in both ratios, although the deviations in the results were fairly large. The quantitative experiments indicated that, in the case of SDS, this was mainly a result of increases in the amounts of PE and PG; the PC amount seemed to be quite stable, which was also observed earlier with TX and SDC [10]. The large amounts of phospholipids (as P) obtained in the presence of CTAB are more difficult to interpret: however, the observed ratios were well in accord with the results obtained with the other detergents studied [10] (see Discussion).

DISCUSSION

As stated earlier [9], the fatty acid compositions of the polar lipids in *P. purpureum* are fairly simple. The characteristic features are the absence of α-linolenic acid (18:3ω3), large amounts of 20:4ω6 and 20:5ω3, and the rare occurrence of C_{16} unsaturated acids, with the exception of *tr* 16:1ω13 in PG; this acid has been found in most plants [16]. Usually it is observed linked with 16:0 so that when the amount of 16:0 decreases, the amount of *tr* 16:1ω13 increases, and vice versa. This seemed not to be the case in *P. purpureum* PG when cultured in the presence of detergents; all tested detergents increased the amounts of both [10].

SDS and CTAB strongly inhibit the growth of *P. purpureum*, which fact is probably partly a result of their inhibition of photosynthesis. It has been stated that detergents uncouple the phosphorylation in mitochondria and chloroplasts even at low concentrations [1]. Basically it seems that all detergent actions on living cells are due to their capacity to bind to membrane proteins and lipids. Fairly large amounts of detergent monomer molecules can be thus bound without disrupting the membrane [2]. SDS binds to both peripheral and intrinsic membrane proteins, which often results in drastic conformational changes and loss of biological activity [1]. Intrinsic proteins, however, are not very readily denatured [5]. Only a few studies are as yet available on the binding phenomena of CTAB [6]; it seems that binding sites for CTAB are highly heterogeneous, so that binding interactions become irregular and complex in nature. The electrical charge in membranes is a very important factor: e.g. the thylakoids, which contain PG, have a negative charge at physiological pH values [2]. Thus a surfactant of opposite charge, such as CTAB, would produce more binding. However, the results indicated that PG was here

Table 3. Percentage amounts of fatty acids in *P. purpureum* PG in the presence of sodium dodecyl sulphate (SDS) and cetyl trimethylammonium bromide (CTAB)†

		Concentration of SDS and CTAB (ppm)			
		SDS		CTAB	
Acid	Control	2.5	5.0	2.5	5.0
12:0	0.3	0.3	tr	1.6	0.3
14:0	1.0	1.2	0.9	2.7	2.2
14:1	0.3	0.9	0.7	0.8	0.8
14:3+16:1	1.4	2.2	1.5	4.0*	1.1
15:0	0.7	1.4	1.6	1.4	2.0*
16:0	26.9	23.7	28.3	23.1	35.8
tr 16:1ω 13	11.8	6.3	12.3	7.6	24.2
16:2ω3	0.8	0.5	0.9	0.4	0.4
16:2ω6	—	tr	0.3	1.0	0.5
16:3ω6	tr	0.3	tr	0.3	0.2
17:0	0.8	0.8	1.2	0.5	0.9
18:0	14.9	5.2*	16.7	11.3	9.6
18:1	5.9	7.5	8.2	5.9	2.6
18:2ω6	2.8	5.2	1.2	2.9	0.4*
18:3ω6	1.3	0.8	0.4	0.5	tr
20:1	tr	1.5	tr	3.4	—
20:2ω9	1.0	7.1	1.3	0.4	1.3
20:3ω6	0.9	3.0	0.6	0.5	—
20:4ω6	10.9	7.9	3.9	4.6	—
20:5ω3	5.4	13.4	2.8	3.1	1.9
22:1	0.9	1.0	1.1	2.1	1.7
23:0	0.7	2.4	0.6	2.2	1.9
24:0	0.8	1.4	6.1	7.8*	5.8
24:1	tr	tr	1.3	0.4	1.6

†See Table 1 for explanations of symbols used.

Table 4. PC/PE and (PC+PE)/PG ratios in *P. purpureum* cultured in the presence of sodium dodecyl sulphate (SDS) and cetyl trimethylammonium bromide (CTAB)†

Detergent	ppm	PC/PE	(PC+PE)/PG
—	0	5.6	39.0
SDS	2.5	0.7**	2.1*
SDS	5.0	0.7**	2.0*
CTAB	2.5	0.5**	3.4*
CTAB	5.0	1.1*	1.9*

Amounts of PC, PE, and PG in *P. purpureum* cultured in the presence of SDS and CTAB as μg P/g dry wt†

Detergent	ppm	PC	PE	PG
—	0	20.0	3.6	0.6
SDS	2.5	30.0	41.2*	34.0
SDS	5.0	23.2	31.6***	27.2*
CTAB	2.5	262.3	485.2	221.3*
CTAB	5.0	189.9*	167.4	187.5

†Average of five independent determinations.

the least susceptible of the studied phospholipids with regard to detergent action. This could possibly be explained by the fact that PG, being located in the thylakoids, is quite inaccessible.

PC and PE expressed more clearly the main effect caused by SDS and CTAB, namely the loss of 20:4ω6 from the fatty acid composition. This can be a result of enhanced phospholipase action, which is known to attack very readily the 20:4ω6 in the 2-position of phospholipids [18]. Usually in natural phospholipids the unsaturated acids are in the 2-position of glycerol [19]. The phospholipase action, in turn, is linked with the incomplete delipidation of the phospholipid exchange protein (PLEP) associated with the presence of detergents [20, 21]. SDS and CTAB form strong complexes (with a negative or positive overall charge, respectively) with the PLEP of PC, disturbing its functions; the PC molecule can then become susceptible to the phospholipase [4].

The results obtained on the PC/PE and (PC + PE)/PG ratios (Table 4) resemble those observed earlier with TX and SDC: all ratios decrease very markedly also in the presence of SDS and CTAB. This decrease seems to be a result of an increase in the absolute amounts of PE and PG. PC has always been found to be quite stable [10]. CTAB caused a great increase in the absolute amounts of all studied phospholipids (determined as P amount). However, the ratios corresponded to those obtained with

SDS and the non-denaturing detergents [10]. This could perhaps be a result of the known ability of CTAB to precipitate mucopolysaccharides and thus to decrease the amount of slime formed by *P. purpureum* [22]. The water-soluble mucilages can form as much as 70% of the dry weight of the cell wall [23], and if this component of *P. purpureum* cells is decreased, the phospholipids may form a seemingly greater part of the dry weight of the cells. The amount and chemical composition of the mucilage depends on the species and on the physiological conditions; usually *P. purpureum* forms very large amounts of mucilage [24].

It is possible to alter the fatty acid pattern of animal membrane constituents, e.g. by diet, but homeostatic mechanisms will limit the changes so as to keep the overall properties of the membrane within certain limits [25]; probably this system also operates in algal cells under chemical stress from detergents. However, cells can resist these changes only to a certain extent. If the fatty acid composition is altered to the more saturated direction, which happens in the presence of detergents, too, cell death occurs when the saturation grade has reached a certain point [25]. These phenomena probably contribute to the observed harmfulness of many detergents towards algal cells, although other influences of the detergents must also be involved in this.

EXPERIMENTAL

Plant material, growth and harvest. The unicellular, halophilous red alga *Porphyridium purpureum* (Bory) Ross strain CCAP 1380/1a (Cambridge, England) was used as a pure culture. Although the alga is widely known under the name *P. cruentum* (Ag.) Naeg., it is more appropriate according to the review in ref. [26] to use the name *P. purpureum*. Growth time was one month on a 1% agar solid medium on Petri dishes under Airam 40W-35 white fluorescent tubes giving on average 100 $\mu E/m^2/sec$ (400–700 nm) 18 hr/day. The tough layer formed by the algae was then quantitatively harvested and lyophilized. The culture medium and other conditions are described earlier [9].

Detergents. Two denaturing type detergents were used: (1) SDS, anionic and (2) CTAB, cationic; for the characteristics of these compounds see, e.g. [1, 27, 28]. SDS is used extensively as a solubilizer in membrane studies and its behaviour is fairly well known [2]. 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 on a silicic acid column [9]. 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) [29] and the phospholipids located as in refs [9, 30]. Extraction from the plates was done using $CHCl_3$–MeOH (2:1). The identification of the studied phospholipids was performed using specific stains [30, 31], commercial standards and 2-D TLC [29]. The PC/PE and (PC + PE)/PG ratios were determined spectrophotometrically [32]. For the quantitative experiments it was assumed that the phospholipids contained *ca* 4% of phosphorus [33].

The PC, PE and PG fatty acid compositions were determined with GC/MS using Me esters as derivatives [34]. The GC/MS analyses and double bond position determinations were performed using methods and instruments previously described [9].

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

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