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BIOSYNTHESIS OF THE CHLOROSULPHOLIPIDS OF *OCHROMONAS DANICA*

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Key Word Index—*Ochromonas danica*; Ochromonadeae; biosynthesis; chlorosulpholipids; acetate; malonate; saturated fatty acids; oleic acid; linoleic acid; chlorination.

Abstract—Radioactively labelled acetate and malonate have been shown to be readily incorporated into the chlorosulpholipids of *Ochromonas danica*. Even-numbered, saturated fatty acids (6C–16C) are also readily incorporated but their efficiency of incorporation increases with increasing chain length. Oleic and linoleic acids are poorly incorporated. Docosane-1,14-disulphate-[13-^{14}C] is readily chlorinated to give mono- to hexa-chloro-derivatives. Evidence is presented to show that chlorination of the chlorosulpholipids is a sequential process.

INTRODUCTION

SULPHUR-CONTAINING lipids were first shown to be present in the golden-brown alga *Ochromonas danica* by Haines and Block.[1] They have since been shown to be present in considerable abundance, constituting over 50% of the sulphur,[2] 15% of the lipids[3] and 3% of the dry weight[3] of heterotrophically-grown, stationary-phase cells. Although Mayers and Haines[4] showed that the structure of one of the sulpholipids was *N*-docosane-1,14-disulphate it was not until 1969 that it was realised that this compound was accompanied by chlorinated derivatives. Haines *et al.*[5] desulphated the sulpholipid mixture by solvolysis and separated the resulting diols by TLC into at least seven components which were subsequently shown to contain different proportions of chlorine; one component was shown to be 13(*R*)-chloro-*N*-docosane-1,14(*R*)-disulphate. Elovson and Vagelos[3] entered this field by chance; in a preliminary study of fatty acid biosynthesis in *O. danica* they found that cell-free extracts of the alga would not only not incorporate malonyl-CoA into long chain fatty acids but also would inhibit fatty acid biosynthesis in other systems. They showed that this inhibitory principle was a family of related substances which included *N*-docosane-1,14-disulphate, its higher homologue *N*-tetracosane-1,15-disulphate and several mono-, di-, tri-, tetra-, penta- and hexa-chloro derivatives, two of which were identified as 13-chlorodocosane-1,14-disulphate and 11,15-dichlorodocosane-1,14-disulphate. They called this new, class of lipids, chlorosulpholipids. Elovson and Vagelos[6] later showed that the hexachlorinated derivative was 2,2,11,13,15,16-hexachloro-*N*-docosane-1,14-disulphate. The only other chlorosulpholipid of the docosane series to be characterized is

[1] HAINES, T. H. and BLOCK, R. J. (1962) *J. Protozool.* **9**, 33.
[2] HAINES, T. H. (1965) *J. Protozool.* **12**, 655.
[3] ELOVSON, J. and VAGELOS, P. R. (1969) *Proc. Nat. Acad. Sci. U.S.* **62**, 957.
[4] MAYERS, G. L. and HAINES, T. H. (1967) *Biochemistry* **6**, 1665.
[5] HAINES, T. H., POUSADA, M., STERN, B. and MAYERS, G. L. (1969) *Biochem. J.* **113**, 565.
[6] ELOVSON, J. and VAGELOS, P. R. (1970) *Biochemistry* **9**, 3110.

2,2,11,13,15-pentachloro-*N*-docosane-1,14-disulphate; however a number have been partly characterized. These are, (i) two trichloro derivatives both having one chlorine distal and two proximal to the secondary alcoholic oxygen and therefore differing in the carbon atoms to which the chlorines are attached, (ii) two tetrachloro derivatives, one having one chlorine distal and three proximal to the secondary alcoholic oxygen whilst the other has two chlorines distal and two proximal, and (iii) a second pentachloro derivative which has two chlorines distal and three proximal to the secondary alcoholic oxygen.

Chlorosulpholipids of the tetracosane series are much less abundant than those of the docosane series and so far the only members to have been wholly or partly characterized are the 14-chloro-, 2,12,14,16,17-pentachloro- and 2,2,12,14,16,17-hexachloro-*N*-tetracosane-1,15-disulphates.[7]

The biosynthesis of the chlorosulpholipids poses a number of interrelated problems. Firstly there is the mechanism of formation of the carbon chain; it is tempting to suggest that the carbon chain arises by way of long chain fatty acids and that the malonyl-CoA pathway and fatty acid elongation systems are involved. Mooney *et al.*[8] have indeed shown that acetate and several fatty acids are incorporated into monochlorinated chlorosulpholipids. Secondly there is the mechanism of incorporation of the sulphate moieties; this involves the identification of, (i) the sulphate donor, and (ii) the stage, during the formation of the carbon chain, at which the two sulphate groups are introduced. Mooney *et al.*[8] have suggested that the sulphate donor is PAPS (3′-phosphoadenosine-5′-phosphosulphate). Mercer *et al.*[9] have shown that a cell-free preparation of *O. danica* is capable of forming PAPS from SO_4^{2-} and ATP which supports this suggestion; however it remains to be proved that the sulphate moiety of PAPS is directly incorporated into the chlorosulpholipids. Thirdly there is the problem of the introduction of the chlorine atoms; this involves, (i) the mechanism of chlorination, (ii) the identification of the stage, during the formation of the carbon chain, at which the chlorines are introduced, and (iii) the determination of whether chlorination is a sequential process. Fourthly there is the question as to whether sulphation precedes or succeeds chlorination. In this paper we report our efforts to elucidate some of these problems.

RESULTS

Incorporation of malonate-[2-^{14}C] into chlorosulpholipids and fatty acids

One litre of medium containing 25 μCi of malonic acid-[2-^{14}C], sodium salt (6·3 mCi/mmol) was given a 10% inoculum of logarithmic-phase, *O. danica* cells and cultured for 30 hr in the light at 25°. The cells were then harvested and the chlorosulpholipid diols and total fatty acids extracted, purified and assayed for radioactivity. An aliquot of the cells was removed for dry weight determination and radioassay. The results (Table 1) show that of the malonate taken into the *O. danica* cells 12·8% was located in the chlorosulpholipid diols and 28·4% in the fatty acids.

Incorporation of acetate-[methyl-$^{14}C^3H_3$] into docosane-1,14-disulphate and myristic acid

One litre of medium containing an appropriate ratio of acetate-[2-^{14}C], sodium salt (56 mCi/mmol) and acetate-[methyl-$^3H_3$], sodium salt (500 mCi/mmol) was given a 10%

[7] HAINES, T. H. (1971) *Progress in the Chemistry of Fats and Other Lipids* (HOLMAN, R. T. M., ed.), Vol. XI, pp. 299–345, Pergamon Press, New York.
[8] MOONEY, C. L., MAHONEY, E. M., POUSADA, M. and HAINES, T. H. (1972) *Biochemistry* **11**, 4839.
[9] MERCER, E. I., THOMAS, G. and HARRISON, J. D. (1974) *Phytochemistry* **13**, to be published.

inoculum of logarithmic-phase *O. danica* cells and cultured for 30 hr in the light at 25°. The cells were then harvested and the chlorosulpholipid diols and total fatty acids extracted and purified. Acetate was well incorporated into the chlorosulpholipid diol mixture; 18% of the ^{14}C administered was found in this fraction. The $^{3}H:^{14}C$ ratio of the diol mixture was determined by liquid scintillation counting and found to be 8·89:1. The diol mixture was then silylated and separated by GLC; the nonchlorinated, docosane-1,14-diol fraction was collected by means of a stream splitter and its $^{3}H:^{14}C$ ratio determined. The fatty acid mixture was methylated and separated by GLC; the myristic acid (C14:0) fraction was collected by means of a stream splitter and its $^{3}H:^{14}C$ ratio determined. The results (Table 2) show very similar $^{3}H:^{14}C$ ratios for docosane-1,14-diol and myristic acid.

TABLE 1. INCORPORATION OF MALONATE-[2-^{14}C] INTO THE CHLOROSULPHOLIPIDS AND FATTY ACIDS OF *Ochromonas danica*

	Radioactivity (dpm)	% Incorporation A	% Incorporation B	Weight (mg)	Sp. act. (dpm/mg)
O. danica cells	5 699 000	10·3	—	3100	1838
Fatty acids	1 619 000	3·1	28·4	104·0	1557
Chlorosulpholipids*	731 700	1·3	12·8	29·7	2550

Ochromonas danica cells were grown for 30 hr in the light at 25° in a medium containing 25 μCi malonic acid-[2-^{14}C]. sodium salt (6·3 mCi/mmol).

A—Percentage incorporation of the [2-^{14}C]malonate administered; B—Incorporation expressed as a percentage of the radioactivity present in the *O. danica* cells at the time of harvesting.

* Chlorosulpholipids were assayed as their diols and include the non-chlorinated forms, docosane-1,14-diol and tetracosane-1,15-diol.

Incorporation of fatty acids-[1-^{14}C] into chlorosulpholipids

Several 1 l. batches of medium, each containing 50 μCi of a different fatty acid-[1-^{14}C], were given a 5% inoculum of logarithmic-phase *O. danica* cells and cultured for 48 hr in the light at 25°. The fatty acids were the even-numbered, saturated fatty acids, C_6–C_{16} and

TABLE 2. INCORPORATION OF ACETATE-[METHYL-$^{14}C^{3}H_3$] INTO THE DOCOSANE-1,14-DISULPHATE AND MYRISTIC ACID OF *Ochromonas danica*

	Radioactivity (dpm) [^{3}H]	Radioactivity (dpm) [^{14}C]	$^{3}H:^{14}C$ Ratio
Total chlorosulpholipids*	179 × 10^6	20 × 10^6	8·99
Docosane-1,14-disulphate†	78 120	6130	12·74
Myristic acid‡	20 100	1580	12·72

Ochromonas danica cells were grown for 30 hr in the light at 25° in a medium containing acetate-[methyl-$^{14}C^{3}H_3$], sodium salt.

* Chlorosulpholipids were assayed as their diols and include the non-chlorinated forms, docosane-1,14-diol and tetracosane-1,15-diol.

† Assayed as the TMS-ether of the diol.

‡ Assayed as its methyl ester.

the unsaturated acids, oleic and linoleic. Hexanoic and octanoic acids were added to the medium as their water-soluble sodium salts. The other fatty acids were each added to the medium as a suspension in 0·3 ml ethanol and 0·2 g of a non-radioactive mixture of chlorosulpholipids; the latter was used as a solubilizing agent. The cells from each batch of medium were harvested and their dry weight and radioactivity determined; all the batches

of cells had very similar dry weights, ranging from 4·25 to 4·97 g (Table 3) and all had incorporated about the same quantity of the labelled fatty acid.

The chlorosulpholipids were then extracted from each batch of cells and solvolysed. The resulting diol mixture was then assayed for radioactivity. Table 3 and Fig. 1 show the incorporation of radioactivity from each fatty acid into the chlorosulpholipid diols expressed as a percentage of the total radioactivity taken up into the *O. danica* cells.

TABLE 3. INCORPORATION OF FATTY ACIDS-[1-^{14}C] INTO THE CHLOROSULPHOLIPIDS OF *Ochromonas danica*

Fatty acid-[1-^{14}C] administered Name	Sp. act. (mCi/mmol)	Dry wt (g) of algae	Amt of fatty acid taken up by algae μCi	μmol	Radioactivity of chlorosulpholipids* μCi	% Incorpn†
Hexanoic (C6:0)	23·6	4·50	5·00	0·21	0·40	8·0
Octanoic (C8:0)	17·0	4·25	5·63	0·32	0·56	9·9
Decanoic (C10:0)	14·3	4·52	6·00	0·42	0·55	9·2
Lauric (C12:0)	31·2	4·97	6·89	0·22	1·03	15·0
Myristic (C14:0)	15·4	4·51	13·72	0·89	2·22	16·2
Palmitic (C16:0)	55·0	4·50	18·36	0·33	3·56	19·4
Oleic (C18:1)	62·0	4·55	11·01	0·18	0·18	1·5
Linoleic (C18:2)	57·0	4·30	11·98	0·21	0·11	0·9

Ochromonas danica cells were grown for 48 hr in the light at 25° in a medium containing 50 μCi of a fatty acid-[1-^{14}C].

* Mixture of the chlorosulpholipid diols including the nonchlorinated forms, docosane-1,14-diol and tetracosane-1,15-diol.

† Incorporation expressed as a percentage of the radioactivity taken up into the algal cells.

Incorporation of docosane-1,14-disulphate-[13-^{14}C] into chlorosulpholipids

One litre of medium containing 3·4 mg of racemic docosane-1,14-disulphate-[13-^{14}C] ($6·8 \times 10^6$ dpm/mmol), synthesized from diethylmalonate-[2-^{14}C] by the method of Mayers *et al.*,[10] was given a 5% inoculum of logarithmic-phase *O. danica* cells and cultured for 30 hr in the light at 25°. The cells were harvested (dry wt 1·52 g); radioassay showed that they had taken up 50·75% (20 300 dpm) of the administered radioactivity. The chlorosulpholipids were extracted, solvolysed and the resulting diol mixture radioassayed (18 500 dpm) and silylated. The TMS-ethers of the diols were separated by GLC and the fractions corresponding to the mono-, di-, tri-, tetra-, penta- and hexa-chlorinated forms of the docosane series of chlorosulpholipids were collected by means of a stream splitter. Each fraction was then assayed for radioactivity. The results are shown in Table 4.

Sequential nature of the chlorination of the docosane series of chlorosulpholipids

One litre of medium in which the sole chloride source was 10 μCi $^{36}Cl^-$ was given a 5% inoculum of logarithmic-phase *O. danica* cells and cultured in the light at 25°. When growth had reached the logarithmic phase the culture was divided into three equal parts, A, B and C, and the cells from each part harvested under aseptic conditions.

The cells from Part A were washed free of $^{36}Cl^-$ with sterile, non-radioactive growth medium and the chlorosulpholipids then extracted and solvolysed. The resulting diol mixture was silylated and the TMS-ethers separated by GLC. The fractions corresponding to the mono-, di-, tri-, tetra-, penta- and hexa-chlorinated forms of the docosane series of chlorosulpholipids were collected by means of a stream splitter. Each fraction was then assayed for radioactivity.

[10] MAYERS, G. L., POUSADA, M. and HAINES, T. H. (1969) *Biochemistry* **8**, 2981.

The cells from Part B were washed free of $^{36}Cl^-$ with sterile, non-radioactive growth medium, then resuspended in 1 l. of the normal growth medium which contains 0·33 g/l. chloride and cultured for a further 24 hr in the light at 25°. The cells were then harvested and subjected to the same analytical procedure as those of Part A.

The cells from Part C were washed free of $^{36}Cl^-$ and sulphate with sterile sulphate-free, $^{36}Cl^-$-free growth medium, then resuspended in 1 l. of the sulphate-free medium and cultured for a further 24 hr in the light at 25°. The cells were then harvested and subjected to the same analytical procedure as those of Part A. The results are shown in Fig. 2.

TABLE 4. INCORPORATION OF DOCOSANE-1,14-DISULPHATE-[13-^{14}C] INTO THE DOCOSANE SERIES OF CHLOROSULPHOLIPIDS OF *Ochromonas danica*

Chlorosulpholipid species: No. of chlorines	Percentage composition*	Percentage radioactivity†
0	24·5	5·4
1	36·9	6·1
2	13·8	6·2
3‡	12·3	5·6
4‡	4·7	10·7
5‡	2·2	15·0
6	5·6	50·8

Ochromonas danica cells were grown for 30 hr in the light at 25° in a medium containing 3·4 mg (400 000 dpm) racemic docosane-1,14-disulphate.

* Percentage composition as determined from GLC peak areas.

† Percentage of the radioactivity in the total chlorosulpholipids of the docosane series.

‡ Two species of each of these species were seen in the GLC trace but were collected as a single fraction for radioassay.

DISCUSSION

Both acetate and malonate appear to be good precursors of the carbon chain of the chlorosulpholipids of *O. danica*; 18% of the labelled acetate administered to the cells and 12·8% of the labelled malonate taken into the cells were incorporated into the chlorosulpholipid diols. This is consistent with the involvement of the malonyl-CoA, fatty acid biosynthetic pathway.

The close similarity of the $^3H:^{14}C$ ratios in the myristic acid and the docosane-1,14-diol isolated from *O. danica* grown in the presence of acetate-[methyl-$^{14}C^3H_3$] suggests that their biosynthetic pathways are very closely related, again implicating, the malonyl-CoA pathway. The $^3H:^{14}C$ ratio of the non-chlorinated member of the docosane series of chlorosulpholipids was selected for comparison with that of a fully saturated fatty acid because its hydrocarbon chain would have undergone least disturbance during its formation in that none of its hydrogen atoms would have been replaced by chlorine atoms. The effect of this replacement on the $^3H:^{14}C$ ratio is seen in the lowered value for the total chlorosulpholipid diols.

Further evidence for the involvement of pathways of fatty acid biosynthesis in the biosynthesis of the chlorosulpholipids comes from the good incorporation of saturated fatty acids into chlorosulpholipid diols. It is apparent from Fig. 1 that as the chain length of

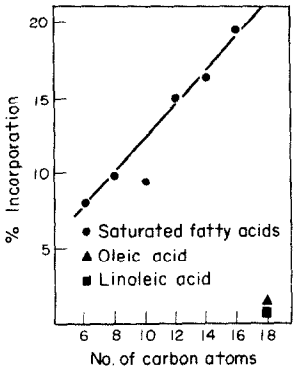


FIG. 1. INCORPORATION OF [1-^{14}C] FATTY ACIDS INTO THE CHLOROSULPHOLIPIDS OF *Ochromonas danica*.

Ochromonas danica cells were grown for 48 hr in medium containing 50 μCi of the [1-^{14}C] fatty acid. All the fatty acids used were equally well absorbed into the alga. The incorporation of each fatty acid into the chlorosulpholipid diols is expressed as a percentage of the total radioactivity in the algal cells.

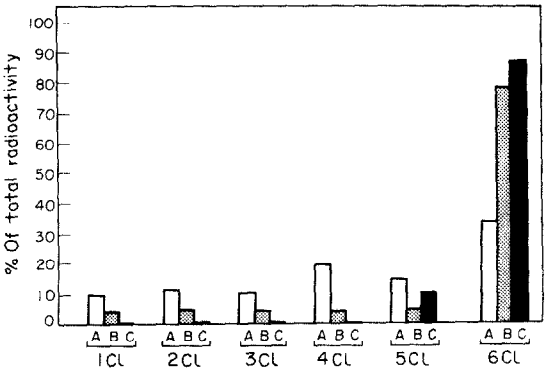
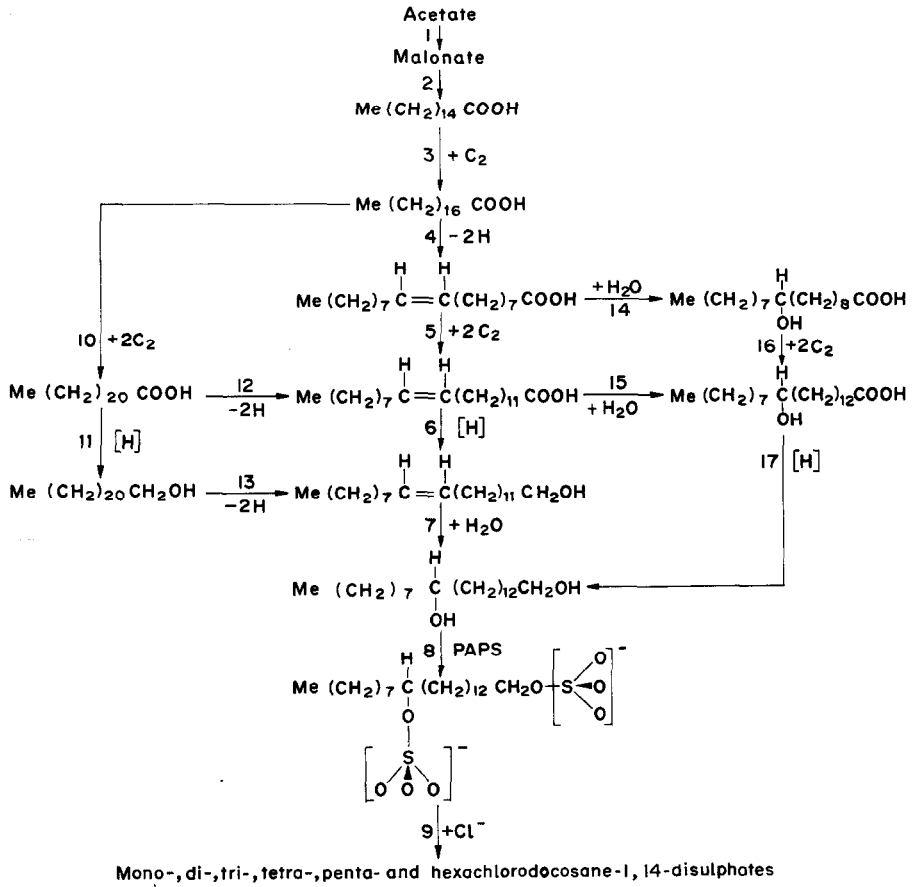


FIG. 2. SEQUENTIAL NATURE OF THE CHLORINATION OF THE DOCOSANE SERIES OF CHLOROSULPHOLIPIDS.

A culture of *Ochromonas danica*, grown to the logarithmic phase in $^{36}Cl^-$, was divided into three equal parts, A, B and C. Part A: the chlorosulpholipids were immediately extracted and solvolyzed. The diols were silylated and separated by GLC. The fractions corresponding to the mono- to hexa-chlorinated forms of the docosane series were collected and radioassayed. Part B: the cells were washed free of $^{36}Cl^-$ and grown for a further 24 hr in normal medium; then treated as were those of Part A. Part C: the cells were washed free of $^{36}Cl^-$ and sulphate and grown for a further 24 hr in a sulphate-free medium; then treated as were those of Part A. The incorporation of radioactivity into each fraction is expressed as a percentage of the radioactivity in the total chlorosulpholipids of the docosane series. Two species of the tri-, tetra- and penta-chlorodocosane-1,-14-diol-TMS ethers were seen in the GLC traces but were collected as a single fraction for radioassay.

the saturated, even numbered fatty acid increases so does its incorporation into the chlorosulpholipids. This is what would be expected if the fatty acids were being elongated by successive addition of 2C units to give a 22C chain. The 18C unsaturated fatty acids, oleic and linoleic acids, however, were very poorly incorporated into the chlorosulpholipids in spite of being absorbed from the medium into the algal cells just as efficiently as were the saturated fatty acids. Mooney *et al.*[8] have shown that octanoate, laurate, palmitate, stearate and oleate are absorbed by *O. danica* cells from the growth medium and are incorporated into chlorosulpholipids. Moreover by degrading the monochlorodocosane-1,14-diol formed when *O. danica* was grown in the presence of these fatty acids, specifically labelled with ^{14}C, they were able to show that the fatty acids were incorporated intact rather than by degradation followed by *de novo* synthesis. However, Mooney *et al.*,[8] apart from showing that palmitate is incoporated into chlorosulpholipids rather better than is stearate, did not examine the relative efficiencies of incorporation of these fatty acids; in particular they did not note the markedly poorer incorporation of oleate. The latter is of particular significance because it tends to suggest that the carbon chain of the chlorosulpholipids is built up by successive elongation of saturated fatty acids to give a fully saturated 22C (or 24C) chain (reactions 3, 10 or 3, 10 and 11, Scheme 1) which is then desaturated (reactions 12 or 13, Scheme 1) and hydrated (reactions 15 or 7, Scheme 1) to give the corresponding diol. This contrasts with the very attractive hypothesis of Mooney *et al.*[8] in which it is suggested that the hydration and subsequent elongation of oleic acid (reactions 14 and 16,

Scheme 1) would give an appropriate hydroxy acid which could then be reduced to the corresponding 22C or 24C diol.



SCHEME 1. POSSIBLE PATHWAYS OF CHLOROSULPHOLIPID BIOSYNTHESIS IN *Ochromonas danica*.

Docosane-1,14-disulphate-[13-^{14}C] is readily absorbed from the growth medium by *O. danica* and is efficiently chlorinated. Over 50% of the label in the total chlorosulpholipids was present in the hexachloro form with 15% in the pentachloro form and significant but progressively lower percentages in the lower chlorinated forms. This experiment does not prove that sulphation *must* occur before chlorination but it certainly shows that it *can* occur before chlorination and, taken with the fact that the non-chlorinated 22C and 24C species are present in considerable amounts in *O. danica*, strongly suggests that *normally* sulphation precedes chlorination. The stage at which sulphation occurs during formation of the carbon chain is unknown but it would seem most likely to occur at the stage shown in Scheme 1 with PAPS as the sulphating agent.

Some light is thrown on the nature of the chlorination process by the results shown in Fig. 2. The chlorosulpholipid diols extracted from the Part A *O. danica* cells had a fairly even distribution of ^{36}Cl, ranging from about 10% in the monochloro species to 34% in the hexachloro species. This must, therefore, have been the distribution of ^{36}Cl in the Part

B cells at the beginning of the 24 hr period of further growth in the presence of non-radioactive chloride. During this period further *de novo* production of chlorosulpholipids could occur since both chloride and sulphate were present in the medium. However those chlorosulpholipids produced would be non-radioactive. Furthermore further chlorination of the lesser chlorinated species formed prior to the final 24 hr growth period would utilize non-radioactive chloride. This would have the effect of increasing the percentage of radioactivity due to ^{36}Cl in the higher chlorinated species and reducing it concomitantly in the lesser chlorinated species. Figure 2 shows that this is precisely what happens; the percentage of radioactivity in the hexachloro species rises to 78·2% whilst that in each of the other chlorinated species falls. A similar but more marked effect is seen in the cells of Part C where 86·7% of the radioactivity is located in the hexachloro species, 10·2% in the pentachloro species and 3·1% evenly distributed amongst the other chlorinated species. These cells were grown for a further 24 hr in a medium containing non-radioactive chloride but no sulphate. Here the *de novo* formation of chlorosulpholipids would be expected to be minimal but further chlorination of lesser chlorinated species would continue. This experiment shows that chlorination is sequential in the sense that the biosynthetic pathway involves a progression from lesser chlorination to greater chlorination. The precise sequence of chlorination is likely to be complex and to occur via a matrix rather than a direct route. The nature of this matrix will not become apparent until the structures of all the members of both series of chlorosulpholipids have been elucidated.

EXPERIMENTAL

Culturing of O. danica. *Ochromonas danica* Pringsheim 933/2 was obtained from the Culture Collection of Algae and Protozoa, The Botany School, Cambridge. It was normally grown in shake culture in the light at 25° on the heterotrophic medium of Aaronson and Baker.[11] Chloride- or sulphate-free media were prepared by replacing the chloride or sulphate salts of the heterotrophic medium[11] by salts having the appropriate anion; L-arginine and L-histidine hydrochlorides were replaced by their parent amino acids. Algal growth was measured turbidimetrically using a Klett-Summerson colorimeter fitted with a Kodak-Wratten 88A filter.

Extraction of chlorosulpholipids. O. danica cells were harvested by centrifugation, washed free of medium, resuspended in a minimal vol dist. H_2O and ruptured in a French pressure cell. The cell crush was extracted 3× using 10 vol. of $CHCl_3$–MeOH (2:1, v/v) on each occasion. The combined extract was shaken with 0·3 vol of 1% (w/v) KCl and the mixture allowed to separate into two phases. The upper, aq. MeOH phase was washed 2× with $CHCl_3$ and the lower, $CHCl_3$ phase was washed 2× with two further 0·3 vol of 1% (w/v) KCl. The $CHCl_3$ washings were combined with the washed $CHCl_3$ phase and reduced to small vol at 40° under reduced pressure for fatty acid analysis. The aq. washings of the $CHCl_3$ phase were combined with the aq. MeOH phase, reduced to 1/3rd of its vol. at 40° under reduced pressure and extracted 3× with 2 vol. *n*-BuOH. The *n*-BuOH extracts were combined and reduced to dryness at 40° under reduced pressure. The residue, containing the chlorosulpholipids, was dissolved in dry MeOH and purified by TLC on silica gel G using $CHCl_3$ MeOH H_2O (10:5:1, by vol) for development. The chlorosulpholipid zones were extracted from the TLC adsorbent, dried by vacuum desiccation, dissolved in dry dioxane and solvolysed[10] to yield the corresponding diols.

GLC of chlorosulpholipid diols. The chlorosulpholipid diol mixture was silylated and subjected to GLC on a 183 cm × 4 mm i.d. glass column packed with 1% SE-30 on 80/100 Gas-Chrom Q. The column oven was programmed to rise from 220° to 280° at 5°/min and then hold. Fractions leaving the column were collected by inserting a 10:1 stream splitter between the column exit and the FID and trapping them in a precooled U-tube loosely packed with glass wool. The GLC fractions were identified by MS.

Analysis of fatty acids. The residue from the $CHCl_3$ extract was saponified in the presence of 0·25% (w/v) pyrogallol as an antioxidant. The saponification mixture was diluted with 4 vol H_2O and the unsaponifiable lipids extracted with Et_2O. The saponification mixture was then acidified (pH 1) with conc. HCl and the free acids extracted with Et_2O. The fatty acids were methylated and separated by GLC on a 183 cm × 4 mm i.d. glass column of 10% SP 1000 on 100/120, acid-washed Chromosorb W. Fractions leaving the column were collected with the acid of a stream splitter (see above).

[11] AARONSON, A. and BAKER, H. (1959) *J. Protozool.* **6**, 282.

Radioassay. Water-soluble materials and lipid-soluble materials were assayed by liquid scintillation counting in NE 250 and NE 216 scintillation fluids (Nuclear Enterprises Ltd., Edinburgh) respectively. The radioactivity of whole algal cells was determined by planchet counting of a thin layer of dried cells.

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