*Phytochemistry*, Vol 23, No 11, pp 2469–2473, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00


# LIPIDS AND LIPID METABOLISM IN THE BROWN ALGA, *FUCUS SERRATUS*

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(*Revised received* 9 *April* 1984)

**Key Word Index**—*Fucus serratus*, Phaeophyceae, brown alga, lipids, fatty acids, lipid metabolism

**Abstract**—The lipids of the brown alga *Fucus serratus* were isolated, identified and quantified The major acyl lipids were the three glycosylglycerides, diacylgalactosylglycerol, diacyldigalactosylglycerol and diacylsulphoquinovosylglycerol These represent over 70% of the total acyl lipids The fatty acid compositions of the major lipids were examined and most showed rather distinctive fatty acid contents For example, diacylgalactosylglycerol was enriched in n-3 polyunsaturated fatty acids while phosphatidylcholine and phosphatidylethanolamine had very high levels of arachidonate Phosphatidylglycerol contained the unusual *trans*-Δ3-hexadecenoic acid The labelling of lipids and fatty acids from [$^{14}$C]acetate was examined and the distribution of label between individual components as a function of the incubation period and in algae collected at different times of the year is reported Algae collected in the winter incorporated much more radioactivity into non-esterified fatty acids when compared to algae collected in the summer All algae could label myristate, palmitate, stearate and oleate at high rates Longer incubation times allowed the labelling of polyunsaturated fatty acids such as linoleic acid

## INTRODUCTION

The marine brown algae represent a very important and prolific class of organisms In spite of this, relatively little is known about their lipid composition in comparison to higher plants or to the Chlorophyceae Lipids comprise *ca* 3% of the dry weight of *Fucus* spp [1] with particularly large amounts of the three glycosylglycerides which are characteristic of photosynthetic organisms—diacylgalactosylglycerol (MGDG), diacyldigalactosylglycerol (DGDG) and diacylsulphoquinovosylglycerol (SQDG) [2] The major phosphoglyceride is phosphatidylethanolamine with smaller amounts of phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol and diphosphatidylglycerol [3, 4] A number of other minor acyl lipids have also been partly identified, some of which have unusual or unique structures [5, 6] The Phaeophyceae are also extremely rich in polyunsaturated fatty acids including octadecatetraenoic, eicosatetraenoic and eicosapentaenoic acids [3, 7]

There has only been one report of experiments on lipid metabolism in Phaeophyceae, it concerned work on labelling of phosphoglycerides from [$^{32}$P]orthophosphate [4] As part of a study of the effect of environment on lipid metabolism and photosynthesis in *Fucus serratus*, we have examined some features of acyl lipid and fatty acid labelling from [$^{14}$C]acetate In addition, we report a complete analysis of the fatty acid composition of individual lipid classes

## RESULTS AND DISCUSSION

Using a method of lipid extraction which we had previously found to be the most effective and reproducible [4], we quantified the individual lipid classes The results are shown in Table 1 It will be seen clearly that the three glycosylglycerides are the major lipid components, as reported in approximate terms before [3] The plant sulpholipid, SQDG, was the major lipid, representing *ca* one third of the total acyl groups *Fucus* spp have been suggested to have one of the highest levels of this particular lipid amongst photosynthetic organisms [8, 9] The two galactosylglycerides were present in *ca* equal molar amounts (Table 1) In contrast, phosphoglycerides were relatively minor constituents (*cf* [4]) The compositional data was confirmed by analysis of phospholipids by phosphate estimation and of glycosylglycerides by sugar measurement and the use of a radiocounting quench method [10]

A major unknown lipid was found which migrated faster than DGDG but slower than MGDG in the TLC systems used It was not labelled by [$^{32}$P]orthophosphate or [$^{35}$S]sulphate and was Dragendorff positive Comparison with authentic diacylglycerol-*O*,*N*,*N*,*N*-trimethylhomoserine (*cf* [11]) showed that it was not this compound To date the unknown lipid has not been fully identified but preliminary data from NMR and mass spectroscopic studies suggest that it may be a dimeric structure of high MW [M Kates and J L Harwood, unpublished results]

Many plant and algal acyl lipids have distinctive fatty acid compositions (*cf* [12]) Therefore, it was of interest to see whether a marine brown alga also showed such characteristics In Table 2 the fatty acid compositions of the major phosphoglycerides of *Fucus serratus* are shown Phosphatidylcholine and phosphatidylethanolamine had a very similar fatty acid content with over 60% of the acyl groups being arachidonate Eicosapentaenoate (n-3) was the next most prevalent fatty acid in these lipids The close similarity in fatty acid patterns for these two phosphoglycerides suggests a biosynthetic relationship, indeed, it is known that the more primitive organisms utilise the methylation pathway for phosphatidylcholine synthesis [13] If such is the case in *F serratus* then it would differ from higher plants where the CDP-base pathway appears

Table 1 Content of acyl lipids in *Fucus serratus*

| Lipid class | Content (μg fatty acid /g fr wt) | Relative proportion % total acyl lipids (g/g fatty acid) | Relative proportion by densitometry (%) | Relative proportion % total phospholipids (lipid-P as % total phospholipid-P) |
|---|---|---|---|---|
| Total acyl lipids | 5622±1571 | | | |
| MGDG | 1018±186 | 18 1±3 3 | 26 7±3 8 | |
| DGDG | 1300±270 | 23 1±4 8 | 8 4±1 5 | |
| SQDG | 1850±359 | 32 9±6 4 | 25 5±4 9 | |
| Acyl-X | 422±219 | 7 5±3 9 | 16 1±0 6 | |
| PC | 242±62 | 4 3±1 1 | 6 3±1 1 | 16 2±2 4 |
| PI | 118±16 | 2 1±0 3 | 7 0±1 8 | 14 4±3 8 |
| PE | 321±79 | 5 7±1 4 | 10 1±1 4 | 36 0±5 2 |
| PG | 141±trace | 2 5±trace | * | 13 0±2 3 |
| DPG | 208±79 | 3 7±1 4 | * | 18 4±3 7 |
| Neutral lipids | 62±22 | 1 1±0 4 | † | |

Lipids were analysed as described under Experimental Results are expressed as means±s d s ($n = 2$ for fatty acid analysis and densitometric analyses and $n = 4$ for phosphate estimations)

*Amounts too small to give sufficient quenching [10]

†Not estimated by this technique due to contamination with pigments Phospholipid phosphorus data is taken from ref [4]

Abbreviations MGDG, diacylgalactosylglycerol, DGDG, diacyldigalactosylglycerol, SQDG, diacylsulphoquinovosylglycerol, acyl-X, see text, PC, phosphatidylcholine, PI, phosphatidylinositol, PE, phosphatidylethanolamine, PG, phosphatidylglycerol, DPG, diphosphatidylglycerol, Neutral lipids, unesterified fatty acids and triacylglycerols

Table 2 Fatty acid composition of the major phosphoglycerides of *Fucus serratus*

| Lipid | Number of estimations | Fatty acid composition (% total acids) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 14 0 | 16 0 | 16 1 (n-9) | 16 1 (n-13) | 18 0 | 18 1 (n-9) | 18 2 (n-6) | 18 3 (n-3) | 18 4 (n-3) | 20 3 (n-6) | 20 4 (n-6) | 20 5 (n-3) | Other |
| PC | 9 | 1 2 | 7 4 | 0 3 | nd | 1 0 | 6 2 | 2 5 | tr | tr | 0 7 | 66 8 | 12 3 | 1 6 |
| | | ±0 8 | ±2 0 | ±0 2 | | ±0 6 | ±1 4 | ±1 4 | | | ±0 5 | ±4 1 | ±1 4 | ±1 4 |
| PI | 7 | 3 5 | 42 3 | 3 8 | nd | 0 5 | 25 6 | 2 8 | 2 5 | 13 1 | tr | 2 6 | 0 1 | 3 2 |
| | | ±1 3 | ±7 2 | ±1 1 | | ±0 4 | ±4 1 | ±1 8 | ±1 5 | ±4 2 | | ±1 1 | ±tr | ±1 8 |
| PE | 9 | tr | 5 4 | 0 2 | nd | 0 3 | 3 5 | 3 0 | tr | tr | tr | 71 9 | 15 4 | 0 3 |
| | | | ±1 8 | ±0 1 | | ±0 2 | ±1 1 | ±1 0 | | | | ±5 5 | ±5 5 | ±0 2 |
| PG | 13 | 0 1 | 13 1 | 0 2 | 1 5 9 | 2 5 | 9 8 | 6 8 | 2 2 1 | 4 3 | 6 6 | 2 7 | 8 6 | 7 3 |
| | | ±tr | ±1 8 | ±0 2 | ±2 3 | ±0 5 | ±1 7 | ±1 7 | ±4 0 | ±1 4 | ±1 8 | ±2 3 | ±4 2 | ±3 3 |
| DPG | 9 | 2 0 | 8 9 | 2 0 | nd | 4 1 | 15 1 | 7 6 | 5 4 | 1 9 | 2 2 | 35 8 | 10 7 | 5 3 |
| | | ±1 0 | ±1 0 | ±0 5 | | ±1 7 | ±2 0 | ±2 5 | ±1 8 | ±0 6 | ±1 3 | ±7 6 | ±4 1 | ±2 6 |

Results are expressed as means ±s e ms with the number of determinations indicated For abbreviations see Table 1, n d not detected, tr, trace (< 0 0 5) Fatty acids are shown with the number before the colon representing the carbon chain length and the figure afterwards representing the number of double bonds Figures in brackets show the position of the first double bond from the methyl end of the carbon chain 16 1 (n-13) contained a *trans* double bond

to be the more important [14] Diphosphatidylglycerol had the same major fatty acids as phosphatidylethanolamine but with a reduced amount of arachidonate and commensurate increases in $C_{18}$ fatty acids Phosphatidylglycerol had a rather distinctive fatty acid composition Firstly, α-linolenate was a major constituent—the only phosphoglyceride where this was so Secondly, the unusual *trans*-Δ3-hexadecenoic acid was a significant component This was of interest in view of current controversy over the possible role of this acid in granal stacking and the organisation of the light-harvesting complex [15, 16] Phaeophyceae such as *F serratus* have chloroplasts which do not contain the usual granal stacks of higher plants Instead, three thylakoid membranes are appressed together [17] They do, however, contain light-harvesting complexes [18] and it would be interesting to know if such proteins contain associated *trans*-Δ3-hexadecenoate as in higher plants [16] Phosphatidylinositol was highly enriched in the saturated fatty acid palmitate (Table 2) and this was consistent with the high proportions of saturated fatty acids found in this lipid from higher plants and Chlorophyceae [12]

Comparison of the fatty acids of the glycosylglycerides

Table 3 Fatty acid composition of glycosylglycerides in *Fucus serratus*

| Lipid | No of samples | Fatty acid composition (% total fatty acids) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 14 0 | 16 0 | 16 1 (n-9) | 18 0 | 18 1 (n-9) | 18 2 (n-6) | 18 3 (n-3) | 18 4 (n-3) | 20 4 (n-6) | 20 5 (n-3) | Other |
| MGDG | 9 | 22 ±11 | 62 ±11 | 08 ±03 | 04 ±02 | 78 ±29 | 70 ±06 | 84 ±22 | 285 ±36 | 52 ±19 | 333 ±14 | 02 ±01 |
| DGDG | 8 | 27 ±18 | 119 ±27 | 18 ±06 | 07 ±03 | 97 ±24 | 63 ±12 | 114 ±18 | 170 ±31 | 117 ±46 | 268 ±50 | tr |
| SQDG | 9 | 25 ±11 | 265 ±60 | tr | tr | 281 ±26 | 87 ±31 | 131 ±32 | tr | 93 ±26 | 69 ±49 | 49 ±20 |

Results are expressed as means ± s e ms For lipid abbreviations see Table 1, for fatty acid abbreviations see Table 2

of *F serratus* (Table 3) showed that the two galactosylglycerides contained large amounts of n-3 polyunsaturated fatty acids In agreement with the data of Jamieson and Reid [7] octadecatetraenoate and eicosapentaenoate were higher in MGDG than in DGDG Also, arachidonate was relatively enriched in DGDG The various percentages of acids in these lipids were in excellent agreement with the previous work [7] We also examined the fatty acid patterns of the glycosylglycerides (and phosphoglycerides) in algae collected at different times of the year but found no significant differences due to seasonal variations This agreed with the rather small changes in overall fatty acid patterns in *F serratus* collected at different times of the year from the Severn Estuary [19] in comparison with algae from the Baltic Sea [2] where temperature variations are much greater The fatty acid content of SQDG showed a considerable enrichment of palmitate and oleate in comparison to the galactosylglycerides Octadecatetraenoate was absent and eicosapentaenoate considerably reduced The relative enrichment of more saturated fatty acids in the plant sulpholipid in comparison with the galactosylglycerides agrees with the situation in higher plants [12]

Radiolabelling studies using [$^{14}$C]acetate as precursor showed that pigments, neutral and polar lipids were all rapidly labelled Uptake of [$^{14}$C]acetate was quite rapid with *ca* 50% of the total radioactivity being taken up by 4 hr and about 90% in 24 hr The distribution of radioactivity in the major non-pigment lipids with incubation time is shown in Table 4 The phosphoglycerides were labelled roughly in proportion to their quantitative importance (Table 1) The glycosylglycerides were rather poorly labelled, with the unknown lipid and the neutral lipid fraction being relatively highly labelled at all times Changes were seen in the relative labelling rates of MGDG and the unknown lipid which contained less radioactivity at 24 hr, and in the neutral lipid fraction which showed an increase between 8 and 24 hr Incubations at shorter time intervals ($\frac{1}{2}$–2 hr) showed a rather similar labelling pattern to 4 hr incubations with regard to the major phosphoglycerides and glycosylglycerides (data not shown) The neutral lipid fraction was, however, less well labelled at short incubation times and it seems to be a characteristic that this fraction is increasingly labelled with time (*cf* Table 4)

The relative labelling of polar lipids from [$^{14}$C]acetate was similar in algae collected at different times of the year (data not shown) However, the distribution of radioactivity within the neutral lipids varied considerably depending on whether algae were collected in the winter or summer months (Table 5) Summer-collected algae accumulated radioactivity mainly into triacylglycerols and sterols In contrast, winter-collected algae, while labelling sterols to the same extent, accumulated radioactivity in non-esterified fatty acids This observation is interesting because it has been observed that seasonally-induced environment changes had a considerable influence on the accumulation of unesterified fatty acids by *Fucus* spp *in vivo* [2, 3]

*F serratus* accumulated radioactivity mainly in saturated and monoenoic fatty acids (Table 6) With time, an increasing amount of unsaturation and elongation was seen, as expected from the normal precursor/product relationships for fatty acid synthesis in photosynthetic organisms [20] In comparison to Chlorophyceae, *F serratus* labelled polyunsaturated acids rather poorly at 15° Only [$^{14}$C]linoleic acid was a significant product although, by 24 hr, radiolabelled α-linolenate and arachidonate could also be detected The proportion of [$^{14}$C]polyunsaturated fatty acids could be increased

Table 4 Time-course of radio-labelling of acyl lipid fractions from [1-$^{14}$C]acetate in *Fucus serratus*

| Lipid fractions | Distribution of radioactivity (% [$^{14}$C]lipids) | | |
|---|---|---|---|
| | 4 hr | 8 hr | 24 hr |
| Neutral lipids | 113±27 | 96±15 | 183±29 |
| Diacylgalactosylglycerol + Diphosphatidylglycerol | 92±09 | 82±08 | 54±08 |
| Phosphatidylethanolamine | 40±06 | 49±05 | 42±10 |
| Unknown lipid | 192±13 | 191±22 | 76±12 |
| Diacyldigalactosylglycerol + Phosphatidylglycerol | 42±12 | 63±15 | 43±07 |
| Phosphatidylcholine | 24±03 | 49±19 | 42±17 |
| Diacylsulphoquinovosylglycerol | 90±07 | 102±10 | 83±09 |
| Phosphatidylinositol | 23±04 | 33±05 | 23±04 |
| Others | 40±11 | 20±03 | 18±03 |
| Number of experiments | 7 | 7 | 8 |

Incubations were carried out in sterilized seawater containing 2 μCi [$^{14}$C]acetate/ml at 20° with 200 μE/m$^2$/sec illumination The lipids were extracted and analysed as described in Experimental Pigments contained *ca* 40% of the total radioactivity in the lipid fraction Results are expressed as means ± s e ms

Table 5 Distribution of radiolabel from [$^{14}$C]acetate in the neutral lipids of *Fucus serratus*

| Sampling time | Incubation time (hr) | No of Samples | % total [$^{14}$C]neutral acyl lipids: Triacylglycerols | Nonesterified fatty acids | Sterols |
|---|---|---|---|---|---|
| Summer | 4 | 5 | 56·4±3·7 | 8·9±3·0 | 34·7±4·6 |
| | 8 | 5 | 62·3±6·9 | 8·6±3·2 | 29·1±3·4 |
| | 24 | 5 | 47·3±4·9 | 8·8±3·0 | 46·7±9·1 |
| Winter | 4 | 5 | 14·1±1·7 | 50·4±4·9 | 35·7±4·3 |
| | 8 | 6 | 16·8±2·9 | 53·2±5·0 | 30·0±4·5 |
| | 24 | 6 | 10·1±1·8 | 52·6±4·0 | 37·3±4·9 |

Algae were collected in the period June–September (Summer) or December–February (Winter) Samples were incubated at 20° with 200 $\mu$E/m$^2$/sec illumination and lipids extracted, separated and quantified as described in Experimental Results are expressed as means ± s e ms

Table 6 Time-course of the radiolabelling of fatty acids from [1-$^{14}$C]acetate in *Fucus serratus*

| Incubation time (hr) | Fatty acid labelling (% total [$^{14}$C]fatty acids): 12 0 | 14 0 | 16 0 | 16 1 | 18 0 | 18 1 | 18 2 | 20 0 | 22 0 | Others |
|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 2 | 6 | 51 | 1 | 20 | 9 | tr | 3 | 6 | 3 |
| | ±1 | ±1 | ±4 | ±1 | ±2 | ±2 | | ±2 | ±1 | ±2 |
| 8 | 1 | 5 | 42 | 1 | 17 | 13 | 2 | 6 | 10 | 3 |
| | ±tr | ±1 | ±3 | ±1 | ±2 | ±2 | ±1 | ±1 | ±1 | ±1 |
| 24 | 1 | 7 | 31 | 1 | 14 | 19 | 6 | 7 | 9 | 5 |
| | ±tr | ±1 | ±4 | ±1 | ±1 | ±1 | ±3 | ±1 | ±1 | ±2 |

Incubations were carried out at 15° with 200 $\mu$E/m$^2$/sec illumination and lipids extracted and fatty acids analysed as described in Experimental Data are expressed as means ± s e ms ($n = 3$) For fatty acid abbreviations see Table 2

considerably by carrying out incubations at lower temperatures such as at 4° [19]

The results reported here show that the lipids of *Fucus serratus* have considerable similarities with those from other photosynthetic organisms, but also some distinctive features The ability of *Fucus serratus* to rapidly incorporate radioactivity from precursors such as [$^{14}$C]acetate into lipids will allow further experiments on lipid metabolism to be carried out These may provide information on the turn-over and function of these lipids in *F serratus* Such experiments will be important because of the distinctive lipid composition of Phaeophyceae and the dearth of knowledge about lipid metabolism in marine algae in general

## EXPERIMENTAL

*Plant material* Healthy samples of *F serratus* were collected from the north side of the Severn Estuary between Lavernock Point and Rhossili throughout the year The tissue was washed with sterilized sea-water (obtained from Rhossili) and kept in sterilized sea-water at 4° with aeration under an illumination of *ca* 40 $\mu$E/m$^2$/sec Control expts showed that the viability of the algae as measured by photosynthetic rates, lipid metabolism and (microscopic) appearance remained good for at least 4 weeks, although the tissue was generally used within 2 weeks of collection Microbial contamination was assessed as previously described [4]

*Incubation conditions* Pieces of tissue (5–7 pieces weighing a total of 0·1–0·2 g fr wt) were cut from the frond tips, washed briefly with 0·5% (v/v) Triton X-100 in sterilized sea water and the detergent removed by successive washes with sterilized sea water The tissues were incubated with 1–4 $\mu$Ci [1-$^{14}$C]acetate in 1 ml of sterilized sea water at the temp indicated in the Table legends in a shaking water-bath Illumination (when required) was 200 $\mu$E/m$^2$/sec (warm white fluorescent tubes)

*Lipid extraction, identification and analysis* At the end of the incubation period, the tissue was removed, thoroughly washed and heated in *iso*-PrOH at 80° for 30 min in sealed tubes Further extraction was continued by the method of ref [21] as detailed in ref [4] Polar lipids were routinely separated by 2-D TLC on prepared silica gel G plates (Merck) using $CHCl_3$–MeOH–$H_2O$ (65 25 4) in the first dimension and $CHCl_3$–$Me_2CO$–MeOH–HOAc–$H_2O$ (10 4 2 2 1) in the second Neutral lipids were separated in a one-dimensional solvent system of petrol–$Et_2O$–HOAc (80 20 1) Routine identification of lipids was by reference to authentic standards after spraying the plates with 8-anilino-4-napthosulphonic acid in MeOH (0·05%) and viewing under UV light Phosphoglycerides were fully identified as in ref [4] and glycosylglycerides and neutral lipids as in ref [22]

Quantification of lipids was by phosphate determination, fatty acid Me ester measurement [4], by sugar estimation [23] and by a scintillation-counting quench method [10] Radioactive compounds were revealed by spark-chamber autoradiography and

individual lipids removed for scintillation counting [24] Correction for quenching was by the external standard channels ratio method

Fatty acids were analysed by methylation of lipid samples using 2 5% $H_2SO_4$ in MeOH at 70° for 2 hr Fatty acid Me esters were separated on a 15% EGSS-X column on Chromosorb W AW (100–120 mesh) (Supelco) at 185° Radiolabelled esters were analysed on similar columns using a gas flow proportional counter Individual peaks were routinely identified by comparison with authentic standards but all major fatty acids were fully identified by GC on polar and non-polar columns, by argentation TLC, by GC/MS (*cf* [22, 25]) and by oxidative degradation [25]

*Acknowledgements*—We are grateful to the Natural Environmental Research Council for financial support and to Professor M Kates, University of Ottawa, Canada for assistance in the preliminary identification of lipids

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