Phytochemistry, 1975, Vol. 14, pp. 623–626. Pergamon Press. Printed in England.

LIPID COMPOSITION OF MATURING AND ELONGATE LIVERWORT SPOROPHYTES

ROBERT J. THOMAS

Thimann Laboratories, University of California, Santa Cruz, CA 95064, U.S.A.

(*Revised Received* 29 *October* 1974)

Key Word Index—*Lophocolea heterophylla*; Hepaticae; liverwort; development; lipids; fatty acids; arachidonic acid; eicosapentaenoic acid.

Abstract—The setae of *Lophocolea heterophylla* sporophytes undergo rapid cell elongation with no net loss of lipid. Glycerolipids and sterol esters are the predominant lipids present in unelongate setae. Phospho- and glycolipids increase dramatically with respect to total lipid during elongation and thus reflect membrane increases. Unusual polyunsaturated fatty acids (arachidonic and eicosapentaenoic) are conspicuous constituents of these lipids.

INTRODUCTION

The Jungermannialean and Metzgerialean liverwort sporophyte consists of a spore capsule, a short stalk or seta, and a foot (the site of attachment to the gametophyte). It is somewhat unique in its ability to undergo rapid, extensive seta elongation by strict cell extension [1]. A 50-fold increase in cell length during elongation is not uncommon [2] and concomitant changes in starch [3], protein [4], and soluble sugars [5] have been studied.

The present investigation was designed to determine the changes in storage and membrane lipids before and after seta elongation. A characterization of the fatty acid composition of seta lipids was also undertaken and is of special interest since many lower green plants are capable of desaturating fatty acids on both the carboxyl and methyl sides of preexisting double bonds [6]. This ability is contrary to the former distinction between pathways leading to polyunsaturated fatty acids in plants (desaturation on the methyl side) and animals (desaturation on the carboxyl). Thus, arachidonic acid, formerly thought to have been a typical polyunsaturated fatty acid of animals, has now been identified in 28 species of bryophytes and 14 species of ferns or fern allies. Among the bryophytes, however, only 2 species of hepatics have so far been investigated [7, 8]. Both are within the order Marchantiales. This study extends these findings to the largest order of hepatics (Jungermanniales), and discusses the possible role played by arachidonic and eicosapentaenoic acids in the sporophytic generation of liverworts.

RESULTS

Table 1 shows the changes in lipid components of *Lophocolea heterophylla* setae extracted at three progressive stages of sporophyte development. In the setae of sporophytes whose spores have undergone meiosis but not yet matured, glycerolipids and sterol esters predominate. They constitute 71% of the total lipid of setae at this stage. In unelongate sporophytes whose spores have matured, the total amount of lipid within the seta is only slightly higher, due apparently to increases in all lipid components except phospho- and glycolipids, free fatty acids and chlorophyll. Glycerolipids and sterol esters still predominate (79%).

There is little difference in size or appearance of sporophytes at either of these first two stages; the only outward indication of spore maturation is a darkening of the capsule. After elongation, the slender seta cylinders are almost translucent in appearance as a result of extreme vacuolation of

PHYTO 14/3 –B

Table 1. Amount of lipids in the setae of developing *Lophocolea heterophylla* sporophytes

Lipid class*	μg fatty acid Me ester/seta†		
	unelongate sporophytes-post meiotic spores, 1 mm‡	unelongate sporophytes-mature spores, 1 mm	elongate sporophytes-mature spores, 25 mm
Total lipid	2·66	3·54	3·59
Phospho- and glycolipids	0·50	0·48	1·20
Monoglycerides	0·23	0·72	0·67
Diglycerides	0·71	0·95	0·35
Free fatty acids	0·25	0·21	0·25
Triglycerides and sterol esters	0·94	1·13	1·06
Chlorophyll§	0·05	0·06	0·06

* Lipid classes were separated by TLC on Si gel using petrol–Et_2O–HOAc (70:30:2). Sterols were also detected on TLC plates and chromatographed very close to diglycerides. † The results are expressed in μg/seta in preference to wet or dry wt since the number of cells in any given seta is constant (at about 4000) and no cell division takes place in the stages under study. μg quantifications were accomplished by comparison of GLC peak areas of constituent fatty acids (without accounting for other moieties) to peak areas of internal standards. ‡ Average seta length. § Spectrophotometric determinations of chlorophyll were performed on total Me_2CO extracts.

cells. Chlorophyll levels in the setae remain unchanged, however, as does the total amount of lipid present. A compositional change does occur with a 2·5-fold increase in phospho- and glycolipids, and a corresponding decrease in diglycerides. Changes in the amounts of other lipid components are not as prominent.

Table 2 shows the fatty acid composition of lipid classes in setae at the three developmental stages. Palmitic, oleic and linoleic acid are the predominant fatty acids in the total lipids. Arachidonic and eicosapentaenoic acids were also detected and were present to varying degrees in all lipid classes (chlorophyll excluded). An increase in both of

Table 2. Fatty acid composition of lipids in the setae of developing *Lophocolea heterophylla* sporophytes

Lipid class*	Developmental† stage	% of total fatty acids‡									
		14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:4	20:5§
Total lipids	post-meiotic	0·7	18·9	2·3	5·3	15·9	29·1	3·9	4·4	6·9	8·6
	mature, 1 mm	2·3	18·8	1·4	4·2	12·0	21·6	8·8	3·4	11·0	11·5
	mature, 25 mm	0·8	24·5	1·6	4·8	10·1	26·2	6·5	3·9	6·3	9·2
Phospho- and glyco- lipids	post-meiotic	1·1	17·4	1·3	0·5	1·6	9·1	3·7	16·6	9·6	23·3
	mature, 1 mm	0·8	30·2	0·8	2·3	15·6	14·4	3·1	0·4	8·1	12·0
	mature, 25 mm	0·1	42·6	2·7	4·4	12·2	22·1	2·2	1·5	0·3	2·8
Mono-glycerides	post-meiotic	0·6	7·8	0·3	3·9	5·9	20·9	4·5	3·6	8·3	24·7
	mature, 1 mm	10·3	10·3	3·1	0·9	4·8	5·5	3·8	0·2	21·4	22·3
	mature, 25 mm	3·0	17·5	0·1	8·0	4·8	6·6	13·9	2·5	13·4	12·1
Diglycerides	post-meiotic		4·2	3·8	4·6	12·4	20·4	7·9	5·8	18·2	7·2
	mature, 1 mm	1·1	6·7	0·5	6·3	15·5	12·5	22·1	0·6	17·3	10·3
	mature, 25 mm		10·9		2·6	1·1	8·2	9·4	0·9	23·7	32·0
Free fatty acids	post-meiotic	2·8	29·6	6·0	11·6	10·1	2·0	2·4		3·1	7·6
	mature, 1 mm		38·0	1·9	11·0	9·5	18·0	1·4	0·5	5·7	12·9
	mature, 25 mm	1·2	27·6	0·4		7·6	11·2	16·0	1·2	5·6	12·0
Tri-glycerides and sterol esters	post-meiotic	0·7	25·6	1·1	5·7	16·3	44·1	1·6	1·0	0·2	2·6
	mature, 1 mm	0·4	24·1	1·3	4·3	11·9	38·8	4·6	0·4	4·7	7·0
	mature, 25 mm	0·7	14·4	2·0	5·5	13·6	49·5	5·0	0·6	4·2	3·0

* Lipid classes were separated by TLC using Si gel and petrol–Et_2O–HOAc (70:30:2). † Post-meiotic and mature refer to the stage of spore development at the time sporophytes were harvested; number of mm refers to the av. length of the setae. ‡ The % composition was estimated from peak areas of fatty acid Me esters separated by GLC on 15% DEGS. § The number before the colon represents the number of carbon atoms in the fatty acid; the number after the colon refers to the number of double bonds present. Small amounts of 16:2, 20:3, 22:0 and 24:0 were found in many samples but are not listed.

these acids during spore maturation, and a subsequent decrease after elongation, is the most significant difference apparent in total lipid fatty acids of setae. There is also a slight increase in overall degree of saturation (from 31 to 36%) following elongation.

Arachidonic and eicosapentaenoic acids are at their highest levels (with respect to total class composition) in mono- and diglycerides and in phospho- and glycolipids. In diglycerides, the proportion of arachidonic and eicosapentaenoic acids increases after elongation; in all other classes and in total lipids, it decreases. The decrease is most striking in the phospho- and glycolipid class. The decreased percentages of these polyenoic acids in phospho- and glycolipids coincides with increases in the percentages of linoleic and palmitic acids. There is a concurrent decrease in linoleic acid of diglycerides, whereas palmitic acid increases in all lipid classes except free fatty acids, and triglycerides and sterol esters. The latter two classes are highest in levels of palmitic, oleic and linoleic acids. Their fatty acid composition more closely approximates that of the total lipids.

DISCUSSION

The total phospholipid content in a tissue can be taken as an approximate measure of the amount of membrane present [9]. Thus the marked increase in phospho- and glycolipids after seta elongation is probably a reflection of corresponding increases in membranes to accommodate for enlargement of seta cells. The increase in phospho- and glycolipids occurs largely at the expense of diglycerides, which decrease following seta elongation. Di- and monoglycerides are characteristically involved as important intermediates in lipid biosynthesis [10].

There is little change in the high level of triglycerides and sterol esters present within *Lophocolea* seta cells following elongation. High levels of triglycerides and sterol esters are also found in moss spores, where they serve as storage lipids [11]. A preliminary experiment on setae from excised sporophytes indicates that the lipid supply is extensively depleted after seta elongation. When the sporophytes remain attached to the gametophyte, which is the normal condition of development employed in this study, the triglycerides and sterol esters are apparently not used as substrates.

The fatty acid composition of total lipids found in liverwort setae is comparable to that found in other lower green plants [10]. Arachidonic and eicosapentaenoic are polyunsaturated fatty acids present in addition to the more typical oleic, linoleic and linolenic unsaturated acids of higher plants. There are high levels of the former in setae from unelongate sporophytes with mature spores. In the field, sporophytes at this stage typically over-winter. A correlation between the presence of arachidonic acid and a capacity for resistance to extreme environmental conditions has previously been suggested [12].

It has also been suggested that arachidonic and eicosapentaenoic acids are specifically associated with (but not restricted to) the chloroplast lipids of lower green plants [12, 13]. Chlorophyll levels in setae remain unchanged, however, after the 3 4 day elongation period. Selective metabolism during seta-cell elongation (which leaves behind the arachidonic and eicosapentaenoic acid containing diglycerides normally required for chloroplast lipid synthesis) could explain why the percentage of these polyenoic acids increases in total diglycerides, and decreases in phospholipids.

EXPERIMENTAL

Lophocolea heterophylla (Scrad.) Dum. was maintained axenically on a modified White's agar medium [14] + 1% sucrose under conditions previously described [15]. Sporophytes at appropriate stages of development were dissected out, intact, from the leafy gametophyte. Prior to extraction, the foot and capsule of each sporophyte were removed.

Lipid extraction. For each analysis, 20–40 freshly dissected setae were extracted and washed by the Folch procedure [16]. Glass microhomogenizers (2 ml) were employed and 0·001% BHT was added to $CHCl_3$–MeOH (2:1) in order to prevent oxidation [17]. Triheptadecanoin (20 μg) was added to each sample as an internal standard for subsequent GLC.

Chromatography. Lipids were spotted onto 0·25 mm Si gel TLC plates and developed with petrol–ET_2O–HOAc (70:30:2) followed by visualization with I_2 vapor or Rhodamine 6G. Lipid classes are identified by standard detection reagents [18] and by comparison of R_f values with reference compounds. Separated lipid classes were eluted from the Si gel with MeOH and 10 μg of a second internal standard, *n*-nonadecanoic acid, was added to each. The constituent fatty acids of the lipid classes were converted to fatty acid Me esters [19] and analysed by FID GLC using a 1830 × 2 mm glass column packed with 15% DEGS on Chromosorb W-HP. The column was maintained at 180°. The injector at 240°. The detector at 255° with a He flow of 35 ml/min. The identity of the compounds was established by comparing R_ts with those of fatty acid Me ester standards and by calculation of ECL values [20]. The % composition of mixtures was estimated from peak areas after correction against blanks; quantitation was accomplished by comparison of peak areas with those of internal standards.

Determination of chlorophyll. Chlorophyll was extracted with 80% Me_2CO and determined spectrophotometrically [21] on a spectrophotometer modified for micro-determinations [22].

Acknowledgements—I am indebted to Dr. R. Donaldson for his advice and assistance with the lipid analysis, to Professors Harry Beevers and William Doyle for use of equipment, and to Professor Doyle and Dr. C. Kelley for constructive criticism of the manuscript. This study was funded in part by an NIH Biomedical Research Support Grant 3-SO5-RRO7135-03.

REFERENCES

1. Askenasy, E. (1874) *Botan. Zeitg.* **32,** 237.
2. Möbius, M. (1915) *Microskopisches Practikum für Systematische Botanik*, Gebrüder Borntraeger, Berlin.
3. Overbeck, F. (1934) *Z. Bot.* **27,** 129.
4. Matthaei, H. (1957) *Planta* **48,** 468.
5. Thomas, R. J. (1974) *Am. J. Botany* **61,** (Suppl.) 12.
6. Fulco, A. J. (1974) *Ann. Rev. Biochem.* **43,** 215.
7. Wagner, H. and Friedrich, H. (1969) *Phytochemistry* **8,** 1603.
8. Gellerman, J. L., Anderson, W. H., and Schlenk, H. (1972) *Bryologist* **75,** 550.
9. Williams, R. D. and Chapman, D. (1970) *Prog. Chem. Fats* **10,** 3.
10. Hitchcock, C. and Nichols, B. W. (1971) *Plant Lipid Biochemistry*, Academic Press, New York.
11. Karunen, P. (1971) *Phytochemistry* **10,** 2811.
12. Anderson, W. H., Gellerman, J. L. and Schlenk, H. (1972) *Lipids* **7,** 710.
13. Wolf, F. T., Coniglio, J. G. and Bridges, R. B. (1966) *Biochemistry of Chloroplasts* (Goodwin, T. W., ed.), Vol. I, pp. 187–194, Academic Press, New York.
14. Hatcher, R. E. (1965) *Bryologist* **68,** 227.
15. Thomas, R. J., Taylor, J. and Montague, M. J. (1970) *Bryologist* **73,** 713.
16. Folch, J., Lees, M. and Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226,** 497.
17. Wren, J. J. and Szczepanowska, A. D. (1964) *J. Chromatog.* **14,** 405.
18. Zweig, G. and Sherma, J. (eds.) (1972) *CRC Handbook of Chromatography*, Vol. II, CRC Press, Cleveland.
19. Lipsky, S. R. and Landowne, R. A. (1963) *Methods in Enzymology* (Colowick, S. P. and Kaplan, N. O., eds.), Vol. VI, pp. 513–537, Academic Press, New York.
20. Jamieson, G. R. (1970) *Topics in Lipid Chemistry* (Gunstone, F. D., ed.), Vol. I, pp. 107–159, Logos Press, London.
21. Bruinsma, J. (1961) *Biochem. Biophys. Acta* **52,** 576.
22. Lowry, O. H. and Bessey, O. A. (1946) *J. Biol. Chem.* **163,** 633.