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METABOLISM OF MOLECULAR SPECIES OF SULPHOLIPID IN BARLEY LEAVES

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Key Word Index—*Hordeum vulgare*; Gramineae; sulpholipid; diacylsulphoquinovosylglycerol; leaf labelling; molecular species; fatty acids; light effect.

Abstract—Molecular species of sulpholipid (diacylsulphoquinovosylglycerol) were separated and analysed after incubation of developing barley (*Hordeum vulgare*) leaves with either (1-^{14}C]-acetate or [^{35}S]-sulphate. The major endogenous molecular species were the trienoic (42%) and the hexaenoic (39%). However, the combined anenoic, monoenoic and dienoic species, which only accounted for 5% of the mass, represented 80% of the labelled species with either precursor. In one experiment, 90% of this radioactivity was found in the dienoic species. The effect of light on the labelling of the molecular species was examined. Acetate is incorporated primarily into the fatty acids of sulpholipid. Transacylation appears to be important in the interconversion of the molecular species of sulpholipid.

The plant sulpholipid (diacylsulphoquinovosylglycerol) is a major constituent of leaf tissue. Although the compound is not just confined either to higher plants or photosynthetic tissues, in leaves it is associated with the chloroplast [1]. In addition, while sulpholipid is concentrated in the lamellar membranes [2] it is not clear whether it is associated with stromal or granal lamellae. Available evidence indicates that it is fairly evenly distributed [3, 4] though X-ray diffraction studies have been interpreted to indicate that it may be associated with photosystem I activity [5]. Non-photosynthetic tissues generally only contain small amounts of sulpholipid but the lipid is present in significant quantities in etiolated tissues [e.g. 6].

The biosynthesis of diacylsulphoquinovosylglycerol in higher plants is, at present, unclear. Most evidence can be explained by a sulphoglycolytic sequence [7–9] but there are certain problems in the detailed mechanism of individual steps [10–12]. The breakdown of sulpholipid in leaf tissue appears to be catalysed initially by a deacylase [13–15]. Further breakdown to sulphoquinovose has been observed in alfalfa [16] and runner bean [c.f. 12]. The degradation in alfalfa appeared to proceed as far as sulpholactaldehyde and sulpholactate [16].

Because of the presence of sulpholipid in the chloroplast and its concentration in the lamellae, it is natural that a role for the compound is photosynthesis should be put forward. Such proposals have included interactions with chlorophyll [17–19], acting as a binding site for phosphoribulokinase [20] and a role in light reaction 1 [21]. Several other suggestions have implicated sulpholipid in various metabolic roles [c.f. 12]. In a study of the labelling of diacylsulphoquinovosylglycerol in broad bean leaves, Heinz and Harwood [22] found that certain molecular species of the lipid were labelled much more rapidly than others. Thus, the major molecular species (1-palmitoyl, 2-linolenyl-) which represented 66% of the total sulpholipid, only contained 30% of the radioactivity incorporated from a number of precursors. In contrast, the monoenoic, dienoic and pentaenoic species were rapidly labelled. These data raised the possibility that diacylsulphoquinovosylglycerol may have more than one function in plants. For example, the slowly-labelled trienoic species may play a structural role while the highly-labelled species may have a metabolic function [12, 22]. As a result of these striking observations with broad bean, we have examined the labelling of molecular species of sulpholipid in more detail using the monocotyledon, *Hordeum vulgare*. The experiments were conducted to determine if the different rates of labelling of molecular species were a general phenomenon, to see if photosynthesis affected the labelling and to find out if there was any evidence for the involvement of sulpholipid in desaturation [c.f. 22].

RESULTS

Kinetics of incorporation of [^{35}S]-*sulphate*

Following a 30 min incubation with [^{35}S]-sulphate, the maximal incorporation of radioactivity was found between 30 and 120 min of a pulse chase. No difference was found in the distribution of radioactivity between molecular species of sulpholipid during the entire period of pulse chase (300 min). A similar result has been reported for [^{35}S]-sulphate and [1-^{14}C]-acetate incorporation in *Vicia faba* [22].

Fatty acid analysis of molecular species of sulpholipid

Sulpholipid from developing cotyledons of barley was separated into molecular species. Analysis of their fatty acids (Table 1) showed that 5% of sulpholipid occurred in the combined anenoic, monoenoic and

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Table 1. Fatty acid analysis of molecular species of diacylsulphoquinovosylglycerol

Molecular species	Fatty acids (% total) 16:0	18:0	18:1	18:2	18:3	SQDG (% total)
Anenoic, mono- and dienoic	75.5 ± 16.7	4.3 ± 3.3	1.2 ± 2.3	11.2 ± 8.3	8.0 ± 9.2	5.3 ± 2.9
Trienoic	36.0 ± 3.1	1.8 ± 0.2	nd	0.2 ± 0.3	62.1 ± 3.3	41.5 ± 14.3
Tetra- and pentaenoic	11.2 ± 2.8	1.8 ± 3.6	nd	23.8 ± 4.3	63.2 ± 4.3	14.3 ± 13.0
Hexaenoic	0.9 ± 0.4	0.4 ± 0.5	tr	nd	98.7 ± 0.7	38.9 ± 19.1

The results are the mean ± standard deviation of 4 replicate analyses. nd: Not detected; tr: trace (< 0.1).

Table 2. Comparison of the effect of light on the incorporation of [1-^{14}C]-acetate or [^{35}S]-sulphate into the molecular species of diacylsulphoquinovosylglycerol

Conditions	Precursor	Molecular species (% incorporation) Anenoic, mono- and dienoic	Trienoic	Tetra- and pentaenoic	Hexaenoic
Light	[1-^{14}C]-acetate	79.4 ± 14.3(5)	12.6 ± 8.2(5)	6.2 ± 3.9(4)	2.1 ± 0.7(4)
	[^{35}S]-sulphate	77.7 ± 6.9(3)	13.3 ± 2.3(3)	1.1 ± 0.2(3)	11.5 ± 0.9(2)
		(ns)	(ns)	(ns)	($p < 0.001$)
Dark	[1-^{14}C]-acetate	79.0 ± 17.6(5)	14.1 ± 17.8(5)	3.9 ± 1.4(4)	3.5 ± 4.2(4)
	[^{35}S]-sulphate	69.0 ± 10.2(3)	17.4 ± 0.6(3)	1.6 ± 0.2(3)	17.7 ± 0.1(2)
		(ns)	(ns)	($p < 0.10$)	($p < 0.02$)
Light vs dark	[^{35}S]-sulphate	ns	$p < 0.10$	$p < 0.10$	$p < 0.001$

ns: Not significant (Student's t test).

dienoic species, approximately 40% occurred in each of the trienoic and hexaenoic species, while the remaining 15% was found in the tetraenoic and pentaenoic species. This distribution accounts for the high content of linolenic acid (73.5%) with palmitic acid (20.9%), the other principal acid.

Effect of light on sulpholipid labelling

Since the sulpholipid of plant leaves is concentrated in the chloroplast lamellae and several lines of evidence have implicated the lipid in photosynthesis, we tested possible effects of light on the labelling of diacylsulphoquinovosylglycerol. Both [1-^{14}C]-acetate and [^{35}S]-sulphate were used as precursors (Table 2). While light stimulated the total uptake of radiolabelled precursor into the leaf, the percentage of radioactivity incorporated into sulpholipid was similar for incubations in the dark and light. The percentage of [^{35}S]-sulphate incorporated ranged from 1 to 4% and that of [1-^{14}C]-acetate varied from 0.02 to 0.94%. The highest incorporations were achieved with the youngest tissues [c.f. 22].

The combined anenoic, monoenoic and dienoic molecular species contained the bulk (about 80%) of the radiolabel incorporated for both precursors and the relative incorporation into these species was not affected by light. The bulk of radioactivity in this fraction was in the dienoic species with the anenoic molecular species only poorly labelled. The percentage incorporation of [^{35}S]-sulphate into the remaining molecular species was significantly reduced by light. This was particularly true for the hexaenoic species. No significant differences were observed between the incorporation of [1-^{14}C]-acetate in the light and in the dark.

When the labelling of different molecular species by by the two precursors was compared, there was a significant difference in the labelling of the hexaenoic species. This species contained a much higher proportion of the total radioactivity from [^{35}S]-sulphate than from [1-^{14}C]-acetate. The converse appeared to be true for the tetraenoic + pentaenoic band, although the differences were only barely significant for incubations in the dark.

Comparison of the fatty acid labelling in individual molecular species

The radiolabelling of the fatty acids of individual molecular species was examined. Radioactivity from [1-^{14}C]-acetate was distributed about 87:13 between the fatty acyl and sulphoquinovosylglycerol portions of sulpholipid respectively. In consequence of this, the relative labelling of the fatty acyl portions of individual molecular species (Table 3) was similar to the results reported above (Table 2) for sulpholipid. No significant label was detected in stearic acid, palmitic acid being the only saturated acid which was significantly labelled. Oleic acid had a particularly high specific activity since it represented only about 0.1% of the total fatty acids

Table 3. Incorporation of [1-^{14}C]-acetate into the fatty acids of molecular species of diacylsulphoquinovosylglycerol

Molecular species	Fatty acid (% incorporation) 16:0	18:1	18:2	18:3	Total labelling %
Monoenoic	22.5	77.5	nd	nd	5.8
Dienoic	8.2	62.6	29.2	nd	68.4
Trienoic	17.0	45.4	15.3	22.3	12.6
Pentaenoic	nd	nd	100	nd	5.2
Hexaenoic	nd	nd	nd	100	2.7

The anenoic (0.5% total label) and the tetraenoic (4.8% total label) species contained insufficient radioactivity for detection of individual fatty acids by radio GLC.

and yet contained over half the total radioactivity. The radioactive dienoic species contained both 16:0/18:2 and di-18:1 combinations and the radioactive trienoic species contained both 16:0/18:3 and 18:1/18:2 combinations. In contrast, the only labelled fatty acid in the pentaenoic species was linoleic.

DISCUSSION

The rapid labelling of the more saturated molecular species of diacylsulphoquinovosylglycerol which was observed first in *Vicia faba* [22] has now been confirmed in leaves from a different plant. When the amounts of sulpholipid distributed in various molecular species were compared (Table 1), it was noticeable that the major fractions (trienoic, pentaenoic and hexaenoic) were all rather poorly labelled. It was suggested [22, 23] that the high labelling of some molecular species and poor labelling of others might be indicative of more than one function for sulpholipid in plant lipids. Rapidly labelled molecular species might have a metabolic function such as with electron transport [21] or phosphoribulokinase [20] while the major molecular species might play a structural role [18]. In view of this possibility the influence of light in increasing photosynthesis was tested (Table 2). When the incorporation of [^{35}S]-sulphate was examined, there was a small decrease in the relative labelling of the more unsaturated molecular species which might have been expected from the above hypothesis. However, the difference was not very marked and the most noticeable effect of light was to increase the uptake of radiolabelled precursor. There was no significant effect of light on [1-^{14}C]-acetate incorporation but this incorporation is complicated by transacylation reactions (see below) and so is more difficult to interpret.

There were small differences in the percentage radioactivity in the pentaenoic and hexaenoic species labelled from the two precursors. Assuming that diacylsulphoquinovosylglycerol is synthesized by a reaction of a nucleoside diphosphate intermediate with diacylglycerol [10, 12], then the above differences could either originate from the radioactivity of the diacylglycerol molecular species or, additionally, via transacylation reactions. Thus, for example, although [^{35}S]-sulphate incorporation may indicate 12–18% of the *de novo* synthesis of sulpholipid is of the hexaenoic molecular species, the poor labelling of linolenic acid from [^{14}C]-acetate would mean that there would be less incorporation from the latter precursor into the hexaenoic species. A similar result was also observed with *Vicia faba* [22]. However, because there was no difference in the pattern of labelling of molecular species from [1-^{14}C]-acetate in the dark and the light whereas there was with [^{35}S]-sulphate, then transacylation must also take place. By that mechanism, for example, the poor labelling of the hexaenoic species from [^{14}C]-acetate is caused by transacylation of poorly labelled linolenic acid to sulpholipid molecules already containing one linolenic acid molecule. Such an acceptor molecule would be the 16:0/18:3-molecular species (see below).

One difference between the labelling of molecular species of sulpholipid in *Vicia faba* and *Hordeum vulgare* was the intramolecular distribution of radioactivity from [1-^{14}C]-acetate. Thus whereas in *Vicia faba* the fatty acids were relatively poorly labelled, they accounted for the bulk of the incorporation in *Hordeum vulgare*. The lack of change in the labelling of molecular species from [^{14}C]-acetate over a 5 hr period tended to eliminate sulpholipid as anything more than a minor substrate for fatty acid desaturation [c.f. 24]. Such a conclusion was also reached for *Vicia faba* [22]. From the labelling patterns observed for fatty acids (Table 3) it is possible to predict likely interconversions between molecular species of sulpholipid. These are indicated in Fig. 1. Key observations are that the saturated and tetraenoic species are very poorly labelled, and that the pentaenoic species only contains radioactivity in linoleic acid. The major molecular species of slupholipid, trienoic and hexaenoic are thus, primarily, each made from only two molecular species. As mentioned above, because of the lack of evidence for desaturation using sulpholipid substrate it seems most likely that transacylation reactions will be responsible for the bulk of the above interconversions (Fig. 1).

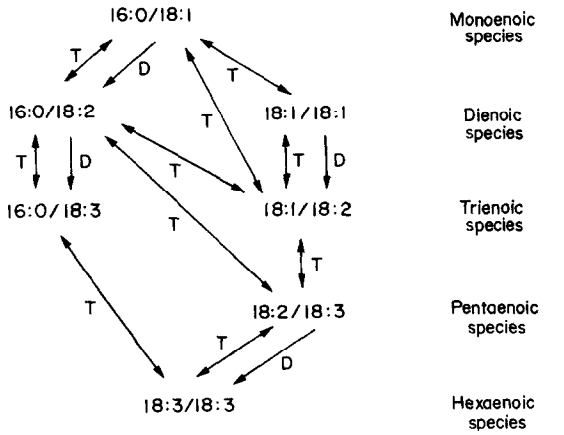

Fig. 1. The principal interconversions for molecular species of sulpholipid. The anenoic and tetraenoic species were poorly labelled, and transacylation of 16:0/18:3 to 18:2/18:3 was precluded by lack of radioactivity in its linolenic acid (Table 3). T; transacylation; D: desaturation. See Discussion for the relative contributions of transacylations and desaturations.

EXPERIMENTAL

Materials. Barley (*Hordeum vulgare* cv Maris Otter) was grown in John Innes Compost at 20° with a 12 hr light period. Material was used 8–10 days after sowing when the leaves were 10–13 cm. Sulpholipid was prepared from spring cabbage (*Brassica oleracea*) as previously described [25]. [1-^{14}C]-Acetate (sp. act. 58 mCi/mmol) and [^{35}S]-sulphate were obtained from the Radiochemical Centre, Amersham, U.K.

Incubations. These were carried out as described previously [22], using 1 or 2 leaves per tube containing up to 10 μCi of isotope in 100 μl of H_2O. The vol. taken up was always < 50 μl/leaf.

Extraction and analysis of sulpholipid. Sulpholipid was extracted by the method of ref. [26]. Samples were removed for chlorophyll and radioactivity determinations. Butylated hydroxytoluene was added to prevent oxidation of fatty acids and the lipid extract separated on Kieselgel G with $CHCl_3$–MeOH–HOAc–H_2O (170:30:20:7). Lipid bands were revealed by spraying with 0.01% aq. Rhodamine 6G, scraped and eluted by successive extractions with $CHCl_3$–MeOH (1:2), $CHCl_3$–MeOH (1:1) and finally $CHCl_3$–MeOH (2:1). The sulpho-

lipid from [1-^{14}C]-acetate experiments was rechromatographed on Kieselgel G with Me_2CO–C_6H_6–H_2O (91:30:8). The position of the isolated sulpholipid was located using a purified standard. Purified sulpholipid was extracted and a sample removed for radioactivity estimation. Fatty acid methyl esters were prepared by heating with 0.5 M MeONa in MeOH at 50°, and were separated on a 2.5 m × 0.7 mm glass column packed with 15% EGSS-X on Supelcoport 100–120 mesh (Supelco Inc., Bellefonte, PA 16823, U.S.A.) at 190° using a Perkin–Elmer F33 gas chromatograph. Radioactive fatty acid methyl esters were separated on similar columns using a Pye 104 gas chromatograph connected to a Panax radioactivity detector.

Separation of molecular species and analysis. Purified sulpholipid was separated into molecular species by TLC on Kieselgel G-$AgNO_3$ (4:1) plates which had been activated for 20 hr at 110° with $CHCl_3$–MeOH–H_2O (65:25:4) at room temp. Improved resolution was obtained by equilibrating the tank and developing the plate at −15°. Individual bands were revealed with Rhodamine 6G and either transmethylated using MeONa or counted directly for radioactivity [9].

Chlorophyll estimation. Chlorophyll was estimated by the method of ref. [27].

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