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LIPID BIOSYNTHESIS BY INTACT MESOPHYLL AND BUNDLE SHEATH CHLOROPLASTS FROM MAIZE

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Abstract—The two dimorphic forms of chloroplast isolated from maize leaves utilized acetate for fatty acid biosynthesis and had similar requirements for cofactors. The oleate:palmitate ratio of the fatty acid products was lower for bundle sheath chloroplasts as was acetate incorporation into total fatty acids. Galactose from UDP-galactose was incorporated into galactolipids by both morphological forms to give monogalactosyl diacylglycerol and digalactosyl diacylglycerol in the ratio of 4:1.

INTRODUCTION

It is well-established that isolated chloroplasts from higher plants synthesise fatty acids from acetate in a light-dependent reaction [1,2] and are able to perform the final step in monogalactosyl diacylglycerol biosynthesis [3]. The recent demonstration by Douce [4] that the envelope of the spinach chloroplast is the site for the incorporation of UDP-[^{14}C] galactose[U] into galactolipids establishes the importance of using isolated chloroplasts with intact envelopes for studies of lipid biosynthesis. Leaves of maize possess two morphologically distinct types of chloroplast in their mesophyll and bundle-sheath cells referred to as "granal" and "agranal" respectively; a recent review by Laetsch [5] discusses the current state of knowledge of the biochemistry and ultrastructure of these types of plastid. Suspensions of chloroplasts containing a high proportion of envelope-bound chloroplasts of both types from young maize leaves, incorporate acetate-[1-^{14}C] into fatty acids [6] and also galactose from UDP-galactose into galactolipids (Hawke and Leese unpublished) but the relationship between capacity for lipid synthesis and the membrane structure of the two morphological types of plastids has not so far been investigated. Differences in the total fatty acid composition of the photosynthetic membranes of the two types of plastid [7] indicate the possibility of different synthetic capacities although the fatty acid compositions of the galactolipid fractions appear to be similar. In addition fragments of bundle sheath chloroplasts have been reported to have higher galactolipid to chlorophyll ratios than intact mesophyll chloroplasts [8].

Anderson *et al.* [9] have published a procedure for maize leaves which separates mesophyll chloroplasts from bundle sheath chloroplast fragments. The present paper describes a modification of this procedure which also yields intact bundle sheath plastids and the results of experiments in which these suspensions and suspensions containing intact mesophyll plastids were compared for their ability to incorporate acetate-[1-^{14}C] into fatty acids and galactose from UDP-galactose into galactolipids.

RESULTS AND DISCUSSION

Acetate incorporation into lipids by intact mesophyll and bundle sheath chloroplasts

Isolated dimorphic chloroplasts prepared from the differentiated leaf tissue of 7-day-old maize plants responded similarly to the omission of cofactors from the standard incubation conditions

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Table 1. Cofactor requirements for incorporation of acetate-[1-^{14}C] into lipids by mesophyll and bundle sheath chloroplasts prepared from maize leaves

Incubation conditions	Incorporation of acetate-[^{14}C] (%) Mesophyll chloroplasts	Bundle sheath chloroplasts
All cofactors	3·4	3·1
−NADH	3·5	3·1
−CoA and −NADH	2·1	1·7
−DTT and −NADH	3·2	2·8
−HCO_3 and −NADH	1·9	2·0

See text for selection of tissue for chloroplast preparation and standard conditions of incubation.

(Table 1) which in earlier work [6] had given optimum acetate incorporation. Cysteine was a component of the isolation medium so that the small effect of dithiothreitol was anticipated. The effect of ATP was examined in separate experiments (Table 2) and again there was a requirement for this cofactor in fatty acid biosynthesis by both mesophyll and bundle sheath chloroplasts. When related to a constant chlorophyll level of 200 μg, mesophyll chloroplasts gave somewhat better acetate incorporation than bundle sheath chloroplasts. Most of the acetate was incorporated into palmitate and oleate in both mesophyll and bundle sheath chloroplasts. However, there was a consistent trend for higher proportions of label to be incorporated into palmitate than into oleate by bundle sheath chloroplasts. Lower activity of both the fatty acid synthetase and desaturases may be a characteristic of bundle sheath chloroplasts or may have resulted from the more vigorous and prolonged procedure required to isolate and purify these chloroplasts. However, the endogenous lipids of bundle sheath chloroplasts contained slightly higher proportions of saturated fatty acids than the lipids of mesophyll chloroplasts. The percentage composition of the major fatty acids were (mesophyll in brackets): 16:0, 24(11); 18:0, 7(1); 18:1, 5(2); 18:2, 7(4); 18:3, 56(80). The incorporation of acetate by both types of chloroplast from 14-day-old plants was considerably lower than by chloroplasts prepared from the distal sections of 7-day-old plants, probably due to greater chloroplast damage occurring during the disruption of the older and more fibrous tissue.

Table 2. Incorporation of acetate-[1-^{14}C] (0·017 μmol; 1 μCi) into fatty acids by mesophyll and bundle sheath chloroplasts isolated from maize leaves

Expt.	Chloroplasts	Incorporation into lipid/200 μg chlorophyll (%)	Acetate incorporation into fatty acids (% of total incorporation) 12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	ratio 18:1/16:0
1*	Mesophyll	1·0			53·1	1·9	12·1	30·0		2·9	0·6
	Bundle sheath	0·8	1·1	2·1	52·0	4·1	10·9	26·0		3·8	0·5
2	Mesophyll	3·6	0·2	2·8	54·7	4·4	7·4	26·0	4·5		0·5
	Bundle sheath	3·2	1·2	5·9	71·5	4·7	5·1	9·5	2·1		0·1
3	Mesophyll	12·6		0·8	30·2	1·1	3·5	58·6	5·7		1·9
	Mesophyll (—ATP)	5·2		5·1	38·0		5·3	46·5	5·1		1·2
	Bundle sheath	4·6 †									
	Bundle sheath (—ATP)	3·8 †		3·7	57·5	4·0		34·8			0·6
4	Mesophyll	7·6	0·3	1·5	48·6	0·8	13·0	30·2	2·6	3·0	0·6
	Mesophyll (—ATP)	4·9		0·3	52·8	0·1	9·7	28·6	0·2	0·3	0·5
	Bundle sheath	5·7	0·8	2·5	46·7	1·0	12·8	30·6	2·7	2·9	0·6
	Bundle sheath (—ATP)	4·2	2·9	4·2	56·1	1·8	13·4	18·4	3·2		0·3
5	Mesophyll	7·6	1·4	1·8	30·9	1·3	3·0	55·4	3·6	2·6	1·8
	Mesophyll (—ATP)	3·6	1·2	1·9	32·4	1·4	3·3	55·5	2·6	1·7	1·7
	Bundle sheath	3·4	2·2	4·2	38·6	6·9	5·6	42·5			1·1
	Bundle sheath (—ATP)	1·2									
6	Mesophyll†	4·3		2·6	37·4	2·2	5·8	46·0	3·3	2·7	1·2
	Mesophyll	4·4		2·9	51·0	3·5	10·3	29·2	3·1		0·6
	Bundle sheath†	4·6		6·4	45·4	3·2	8·7	27·1	9·2		0·6
	Bundle sheath	6·0		3·8	53·4	4·7	10·2	22·8	5·1		0·4

* Chloroplasts prepared from 14-day-old plants; † incubation at 15°.
Fatty acid analysis carried out on combined sample.

As found in earlier experiments with chloroplasts isolated from maize [6] and other higher plants [10], little synthesis of 18:3 occurred. Lowering the temperature of incubation from 20 to 15° raised the oleate:palmitate ratios in the newly-synthesized fatty acids of both types of chloroplast. This effect of temperature on desaturation appears to be a general phenomenon and has been observed in whole plants [11], plant tissue [12,13] and isolated mesophyll chloroplasts [10]. Similar changes occurred with both mesophyll and bundle sheath chloroplasts when ATP was omitted (Table 2).

Utilization of UDP-[^{14}C] gal[U]

UDP-galactose–diglyceride galactosyl transferase which catalyses the final step in monogalactosyl diacyglycerol biosynthesis appears to be a comparatively stable enzyme since it remains highly active during the preparation of acetone powders of spinach chloroplasts [3,14]. Consequently activities of this enzyme from the two chloroplastic sources are less likely to be affected than the fatty acid synthetase by the differential grinding procedures used to prepare mesophyll and bundle sheath chloroplasts.

It was found that the rates of incorporation of galactose from UDP-galactose into galactolipids by the two types of chloroplast were very similar and after 40 min about 22% of the label had been utilized for galactolipid synthesis. The rates of incorporation from these and other experiments were about 10-fold lower than those obtained by Douce [4] using spinach chloroplasts. The similarity of galactolipid synthesis in mesophyll and bundle sheath chloroplasts was also indicated by three other experimental observations. No age differences in synthetic capacity were apparent because about 20% galactose from UDP-galactose was incorporated into galactolipids in 30 min incubations by both types of chloroplasts from both 7- and 14-day-old tissue. In addition, the ratio of monogalactosyl diacylglycerol to digalactosyl diacylglycerol synthesized was about 4:1 with both types of chloroplast suspensions, which accords with the constant ratio found for endogenous levels [8]. Thirdly, the substitution of Tris–HCl buffer by the sorbitol–tricine buffer used in the incubations with acetate as substrate resulted in a five-fold reduction in galactose incorporation in both bundle sheath and mesophyll chloroplasts.

It may be concluded that the mechanism for fatty acid and galactolipid biosynthesis in the mesophyll and bundle sheath chloroplasts of maize have essentially the same characteristics.

EXPERIMENTAL

The differential grinding procedure described by Anderson *et al.* [9] was modified for the preparation of intact mesophyll and bundle sheath chloroplasts from maize leaves grown for 7 days in an artificial environment [15]. Only tissue in which differentiation of chloroplasts into the two dimorphic forms had occurred was used as a source of chloroplasts, (tissue more than 8 cm from the base of the maize leaves in 7-day-old plants [15]) In one experiment 14-day-old plants were used. The isolation medium consisted of 0·5 M sucrose in 0·067 M Pi buffer at pH 8, 0·2% bovine serum albumin (Cohn Fraction V), 1 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$ and 5 mM cysteine. In the modified method, weighed and prechilled leaves were cut into 2–3 mm wide strips, and blended into isolation medium in an Atomix for 4 sec at 38% of the line voltage. The brei was filtered through two layers of Miracloth, the filtrate centrifuged for 30 sec at 300 g, and the supernatant retained. This was centrifuged for 90 sec at 3000 *g*, and resulting pellet resuspended and layered onto a gradient consisting of 20 ml of isolation medium in which 0·6 M sucrose replaced 0·5 M sucrose, and then centrifuged for 15 min at 440 *g* [15] to yield a pellet of purified mesophyll chloroplasts. This isolation medium and purification technique gave maize chloroplast preparations showing good acetate incorporation into long chain fatty acids [6].

Residue from the above filtration was resuspended in isolation medium, homogenized at 75% of the line voltage for 1 min, 20 and 15 sec successively, filtered after each homogenisation, and combined filtrates discarded in an attempt to remove any remaining mesophyll cells. Fibrous residue consisting of bundle sheath cells was then washed, filtered and chopped with razor blades in a small vol. of isolation medium to release bundle sheath chloroplasts and filtered through 2 layers of Miracloth. Chopping procedure was repeated × 3 and the filtrates combined and filtered together through 10 layers of 25 μm nylon bolting cloth. The final filtrate was centrifuged for 5 min at 3000 *g* and the pellet resuspended in 0·5 M sucrose and the bundle sheath chloroplasts collected after centrifugation through 0·6 M sucrose.

We were able to distinguish between mesophyll and bundle sheath chloroplasts by phase contrast microscopy [9], and to assess the homogeneity of the preparations by electron microscopy as described by Leese *et al.* [15]. Mesophyll chloroplast preparations were virtually completely freed from contamination with cell debris, nuclear material, mitochondria and bundle sheath chloroplasts, and approximately 40–50% of the isolated mesophyll chloroplasts had intact outer envelopes. In the suspension of bundle sheath chloroplasts, the majority of the bundle sheath chloroplasts were intact, but the preparations was contaminated to the extent of approximately 25% with broken mesophyll chloroplasts. The bundle sheath chloroplast preparation contained more cellular contamination than the mesophyll preparation, in particular nuclear material.

The reaction mixture for investigating acetate-[1-^{14}C] incorporation into fatty acids contained 50 mM Tricine buffer

at pH 7·8, 300 mM sorbitol, 50 mM Pi at pH 7·8, 2 mM ATP, 0·5 mM CoA, 30 mM $NaHCO_3$, 2·5 mM dithiothreitol, 0·5 mM $MgCl_2$, 0·2 mM NADH or NADPH, 0·017 μmol (1 μCi) acetate-[1-^{14}C]. Chloroplasts were added to the reaction mixture as a suspension in isolation medium. Final vol. was adjusted to ml and the mixture incubated with shaking at 20° in light (25000 lx). Incubation of chloroplasts with UDP-[^{14}C] Gal[U] (0·214 nmol and 0·05 μCi) was carried out at 30° for 40 min (with no special light requirement) in 0·1 M Tris–HCl buffer at pH 7·4 using a total vol. of 0·2 ml, or in the reaction mixture given above for acetate incorporation. The methods of extraction of lipid, isolation of the lipid fractions and the determination of the radioactive products following incubation with acetate-[1-^{14}C] and UDP-[^{14}C] Gal[U] respectively have been described [14]. The incorporation of radioactive substrates by chloroplasts in each of the experiments is adjusted to a chlorophyll content of 200 μg. Chlorophyll and chlorophyll *a/b* ratios were determined by the method of Arnon [16]. In agreement with Andersen *et al.* [17] who found that the chlorophyll *a/b* ratio in mesophyll and bundle sheath chloroplasts did not differ greatly until about 9 days after sowing, we found the ratios in bundle sheath cells and chloroplasts only slightly higher than in mesophyll chloroplasts. Typical values for chlorophyll *a/b* ratios were: whole leaf, 3·3; mesophyll chloroplasts, 3·1; bundle sheath cells, 4·3; bundle sheath chloroplasts, 3·6.

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