On the Occurrence of Monoacylglycerol Derivatives in Lipid Metabolism of Chloroplasts

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Z. Naturforsch. **37 c,** 218–225 (1982); received November 26, 1981

Monoacyllipids, Chloroplast Lipid Synthesis

The aim of this investigation was to elucidate the rapid synthesis of monogalactosyl monoglycerides and their role as intermediary acyl-acceptors in galactolipid synthesis of spinach chloroplasts. The problem was attacked by studying the incorporation of sn-[^{14}C]glycerol-3-phosphate and [1-^{14}C]acetate into the lipid fraction of gently shocked and reconstituted preparations. The data revealed:

1. a concurrent accumulation of both monoglycerides and monogalactosyl monoglycerides under acidic incubation conditions, with C_{16}-fatty acid species predominating,
2. similarities in the fatty acid incorporation of both monoacyllipids,
3. the occurrence of two isomeric forms viz. 1- and 2-O-acyl-isomers of these lipids.

Thus, it appears that monogalactosyl monoglycerides are synthesized by galactosylation of monoglycerides rather than by galactolipase hydrolysis.

Both monoacyllipids are likely to be derived from the corresponding lysophosphatidic acids by dephosphorylation. Their fatty acid incorporation pattern therefore may contribute to an understanding of the specific esterification of different fatty acids at the C_1- and C_2-position of the glycerol moiety of galactolipids. Analysis of the specific acylation of monoglycerides and monogalactosyl monoglycerides as well as the nature of the acyl donors involved in this fatty acid transfer yielded the following observations:

5. The position of the fatty acids within the monoacyllipids seems to depend on whether acyl-ACP or acyl-CoA is the primary acyl donor.
6. The characteristics of the fatty acid incorporation into monoglycerides and their galactosylated derivatives support the notion that a successive acylation of sn-glycerol-3-phosphate occurs first in the C_2- and then in the C_1-position.
7. In contrast, the chain length of the fatty acids incorporated seems to be determined by such factors as pH and the concentration of sn-glycerol-3-phosphate. This observation suggests that these parameters may act by controlling the elongation of ACP-bound C_{16}-fatty acids to their C_{18}-species.

Introduction

Monogalactosyl diglycerides are synthesized in plants by galactosylation of diglycerides in the chloroplast envelope [20]. Monogalactosyl monoglycerides (MGMG) on the contrary have been thought to be principally formed by lipase hydrolysis of monogalactosyl diglyceride [1]. Furthermore the existence of enzymes catalyzing the reverse reaction, *i.e.* the reacylation of MGMG, has been demonstrated [2] in chloroplasts. We have previously reported that trace amounts of a monogalactosylmonoglyceride isomer, called I-MGMG, are rapidly synthesized and subsequently acylated to monogalactosyl diglycerides during chloroplast lipid synthesis *in situ* [3, 4]. This rapid synthesis suggested that lipase hydrolysis is excluded and a formation through galactosylation of monoglycerides is possible, although final evidence was still lacking. The two pathways to MGDG are depicted in Scheme 1.

Glycerol-3-phosphate-acyltransferase, which catalyzes the acyl-transfer from long-chain thioesters (acyl-CoA or acyl-ACP) to sn-glycerol-3-phosphate, appears to play a role in determining which branch of the pathway to MGDG is used. The type of long chain thioesters (acyl-CoA or acyl-ACP) seems to determine the position of the hydroxyl group being esterified [5, 6] and to influence in this way the further fate of the particular lysophosphatidic acid.

This paper attempts to establish and clarify further the importance and the circumstances under

Abbreviations: ACP, acyl carrier protein; CoA, coenzyme A; DG, diglyceride; LPA, lysophosphatidic acid; 1-MG and 2-MG, monoglyceride isomers; MGDG, monogalactosyl diglyceride; I-MGMG and 2-MGMG, monogalactosyl monoglyceride isomers (lyso-MGDG); PA, phosphatidic acid.

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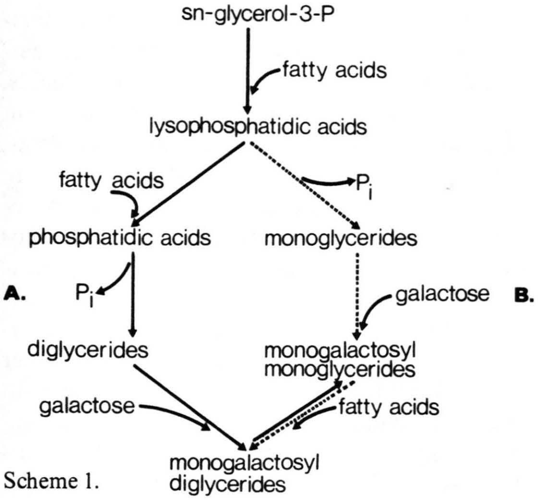

Scheme 1.

which the alternate path of MGMG synthesis through monoglycerides is operative. Our starting point is the acyltransferase specificity and an assessment of accumulation and turnover of monoglycerides under various experimental conditions. The extrapolation of the findings obtained with cell free systems to the intact plant organism will be discussed. The findings will be finally compared to the above mentioned scheme.

Materials and Methods

Spinach (*Spinacia oleracea* L.U.S. Hybrid 424, Ferry-Morse Seed Co.) was grown in water culture [7]. [1-^{14}C]acetate (58.2 mCi/mmol) and sn-[^{14}C]glycerol-3-phosphate (171 mCi/mmol) were purchased from Amersham, Braunschweig. Biochemicals and enzymes were from Boehringer Mannheim, with the exception of Coenzym A (trilithium salt from yeast), oleoyl-CoA, palmitoyl-CoA and the fatty acid standard mixture for gaschromatography, which were obtained from Sigma.

Chloroplasts were isolated from spinach using conventional techniques (tricine-NaOH/sucrose medium) and rapidly purified according to the method of Mills and Joy [8]. ^{14}C-incorporation into chloroplasts was carried out

1. in the leaf homogenate (times and conditions of incubation are noted in the legends to figures and tables) prior to the isolation of the chloroplasts [3].
2. after isolation and purification of the chloroplast.

When intact, purified chloroplasts were utilized, they were broken before use, by gentle osmotic shock in 10 mM buffer media (MES, pH 6.5 or tricine pH 8.0) for the times indicated in order to be sure that the envelope was not a barrier for added cofactors. The *standard reaction mixture,* if not otherwise defined contained: buffer medium (MES pH 6.5 or tricine pH 8.0), 10 mM; sucrose, 0.3 M; ATP, 4 mM; CoA, 0.5 mM; MgCl$_2$, 1 mM; sn-glycerol-3-phosphate, 1 mM and known amounts of chloroplast extracts and/or chloroplast membranes. However, when oleoyl-CoA or palmitoyl-CoA was added to the reaction mixture, ATP and CoA were omitted. Chloroplast *envelope membranes* as well as *chloroplast extracts* (stroma) were prepared from intact purified spinach chloroplasts according to Joyard and Douce [9]. Separation and *analysis of long-chain thioesters* were as described by Mancha *et al.* [10]. *Lipids* were extracted according to Bligh and Dyer [11] and chromatographed in two dimensions on silica gel coated thin layer plates (Macherey and Nagel) – 1st elution: acetone/benzene/water = 91 : 30 : 8 v/v/v; 2nd elution: petroleum ether (40–60)/diethylether/formic acid = 60 : 40 : 1.5 v/v/v. This method permits a sufficient separation of the investigated lipids as well as the discrimination between 1- and 2-monoglycerides (1-MG and 2-MG). While both lipids were found at the same R_f-value after the first elution, only 2-MG showed a slight mobility in the second (apolar) solvent system. The discrimination between 1- and 2-monoglycerides was carried out on silica gel impregnated with boric acid according to Thomas *et al.* [26]. The simultaneous occurrence of two monogalactosyl monoglycerides [4] in chloroplasts suggested their structural and synthetic relationship with the 1- and 2-monoglycerides. In the present paper we therefore distinguish between 1- and 2-monogalactosyl monoglycerides (1-MGMG and 2-MGMG), according to their polarity, although the direct demonstration of the 2-O-acyl-isomer failed as yet [4]. Radioactive lipid spots were located by scanning, scraped into counting cartridges and vigorously shaken in 2 ml of methanol. Afterwards 10 ml of scintillation fluid (6 g butyl-PBD, 600 ml toluene, 400 ml methylglycol) were added and samples counted by liquid scintillation. Fatty acid analysis by GLC was described by Heise and Jacobi [12]. ^{14}C-labeled fatty acid methylesters were collected in an automatic fraction collector (Packard Instruments Co.) and the contents of the vials were counted by liquid scintillation as described above. Protein concentration was determined by the method of Lowry *et al.* [13].

Results

The significant increase of monogalactosyl monoglyceride formation in weakly acidic chloroplast suspensions, shown in our recent investigations [4], appeared to be connected with the monoglyceride accumulation in cell free systems, described by Joyard and Douce [9]. Therefore, following these authors, glyceride synthesis from sn-[^{14}C]glycerol-3-phosphate by purified and gently shocked spinach chloroplasts (with oleoyl-CoA (0.1 mM) as acyl donor) was compared under weakly acidic (pH 6.5) and alkaline (pH 8.5) conditions. The data (Fig. 1) confirm the expected monoglyceride accumulation. Moreover accumulation is significantly increased at pH 6.5. The monoglyceride isomer predominantly formed under the conditions described is the 1-monoglyceride. Lysophosphatidic acid synthesis in contrast is identical at both pH-values (pH 6.5 and 8.5) and shows the expected precursor function in monoglyceride formation. The synthesis of the secondary acylation products of sn-glycerol-3-phosphate is significantly reduced under the conditions described. The same experiment was repeated and extended, this time using labeled fatty acids. In order to avoid the complex cofactor requirement for the polar lipid synthesis of isolated chloroplasts, described by other authors [6], chloroplasts were labeled with [1-^{14}C]acetate in the spinach leaf homogenate prior to isolation and purification. Such chloroplasts were then incubated with sn-glycerol-3-phosphate at different pH values.

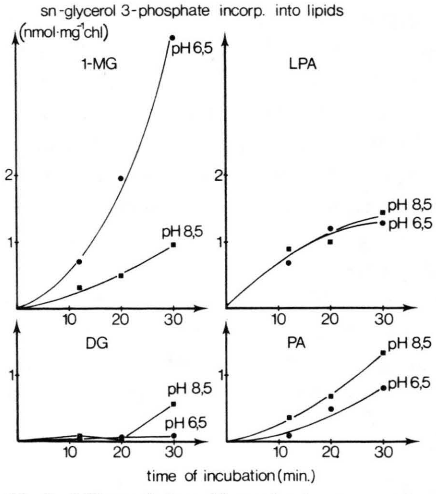

Fig 1. Effect of the pH on the incorporation of sn-[^{14}C]glycerol-3-phosphate into lysophosphatidic acid (LPA), phosphatidic acid (PA), 1-monoacylglycerol (1-MG) and diacylglycerol (DG) by gently shocked chloroplasts (purified on a 40% Percoll gradient). The modified standard incubation medium (see Methods) contained sn-glycerol-3-phosphate (0.5 mM, specific activity: 12 mCi × mmol^{-1}) and a single long-chain thioester (0.1 mM oleoyl-CoA) as acyl donor.

As shown in Table I the amount of the predominantly formed radioactive monoglyceride isomer (1-MG) is dependent on pH. This is not seen in the DG intermediate. In contrast to the preceding experiment a lower amount of label in MG compared to DG is observed. This may be due to the more rapid turnover of lysophosphatidic acid to phosphatidic acid, possibly because other thioesters are supplied for this reaction [5, 6]. The analysis of the esterified fatty acid species reveals the preferential incorporation of palmitic and oleic acid, as has been earlier described by other authors [15]. As shown by the [^{14}C]oleate/[^{14}]palmitate – ratio (Table I a), in DG and MGDG the icorporation of palmitic acid is favored. In the two mainly labeled monoacyl lipid isomers (1-MG and 2-MGMG) this incorporation ratio seems to depend on the pH. Comparison of the incorporation of unsaturated C$_{18}$- and C$_{16}$-fatty acids shows an accumulation of unsaturated C$_{16}$-acids in 1-MG and 2-MGMG under weakly acidic conditions. MGDG in contrast incorporates unsaturated C$_{18}$-fatty acids as can be inferred from a comparison of the sum of unsaturated C$_{18}$- and C$_{16}$-acids in Table I b. This observation leads to the question whether, at low pH, MGDG is formed by introducing C$_{16}$-acids into its lyso compound (2-MGMG), which afterwards is further acylated to MGDG with unsaturated C$_{18}$-acids. To answer this question "[1-^{14}C]acetate labeled chloroplasts" (as in the preceding experiment) were used in a chase experiment. The light chase (20 min) was performed under weakly acidic conditions. In contrast to the preceding experiment, cofactors like sn-glycerol 3-phosphate, CoA and ATP were omitted.

Fig. 2 (A + B) shows, that in MGDG the incorporation of C$_{16}$-fatty acids, when compared to C$_{18}$-acids, is significantly favored. In contrast the fatty acid moieties of the monogalactosyl monoglyceride isomer (2-MGMG) show a relative stable labeling during the incubation period, being nearly equally

Table I. Effect of the pH on the distribution of labeled fatty acids (from [1-^{14}C]acetate) in some lipids from gently shocked chloroplasts. The chloroplasts were already pulse-labeled with [1-^{14}C]acetate in the spinach leaf homogenate (composition: Tricine/NaOH, 50 mM, pH 7.8; sucrose, 0.33 M; EDTA, 2 mM; $MgCl_2$, 1 mM; [1-^{14}C]acetate, 69 μM (specific activity: 58.2 mCi × mmol^{-1}); Chl, 200 μg/ml – conditions: 25 °, saturating white light, mechanical shaking 30 min prior to isolation (centrifugation: 2 min at 1000 × *g*) and purification (2 washes in the same medium, free of label). After a gentle osmotic shock in 10 mM buffer medium (tricine/NaOH, pH 8.5 or MES, pH 6.5) for 10 min, aliquots of the chloroplasts were incubated for 30 min in a reaction micture containing: buffer medium, 10 mM (tricine/NaOH, pH 8.5 or MES, pH 6.5); sucrose, 0.33 M; $MgCl_2$, 1 mM; sn-glycerol-3-phosphate, 1 mM; ATP, 4 mM; CoA, 0.5 mM and Chl, 400 μg/ml (final volume 2 ml).

Lipid	pH	[1-^{14}C]acetate incorporated into lipids (nmol · 10^{-1} mg^{-1} Chl)	Distribution of radioactivity (from [1-^{14}C]acetate between C_{18}- and C_{16}-fatty acids, given as ratio)	
			a. [^{14}C]oleate / [^{14}C]palmitate	b. Sum of unsaturated [^{14}C]-C_{18}-fatty acids* / sum of unsaturated [^{14}C]-C_{16}-fatty acids
1-MG	8.5	1.5	1.2	2.9
	6.5	2.7	0.7	1.5
DG	8.5	7.6	0.5	5.6
	6.5	8.2	0.6	5.1
2-MGMG	8.5	0.4	1.0	3.7
	6.5	0.6	0.8	0.8
MGDG	8.5	4.0	0.5	3.2
	6.5	3.1	0.7	3.0

* Monoenoic, dienoic and trienoic fatty acids are summarized.

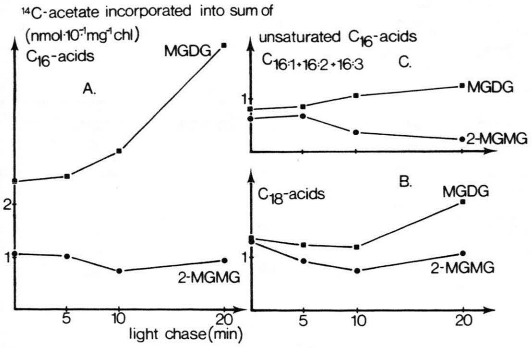

Fig. 2. Distribution of radioactivity (from [1-^{14}C]acetate) in the fatty acid residues of MGDG and 2-MGMG from spinach chloroplasts during a chase in saturating white light under weakly acidic conditions (pH 6.5). The chloroplasts were already pulse-labeled with [1-^{14}C]acetate in the spinach leaf homogenate (composition: Tricine/NaOH buffer, 50 mM, pH 7.8; sucrose, 0.33 M; EDTA, 2 mM; $MgCl_2$, 1 mM; [1-^{14}C]acetate, 0.34 mM (specific activity: 58.2 mCi × mmol^{-1}); Chl, 0.2 mg/ml; – conditions: 25 °, saturating white light, mechanical shaking) 15 min prior to isolation (centrifugation: 2 min at 1000 × *g*) and purification (2 washes in the same medium, free of label). The following light chase was under the same conditions, except of an acidic pH (MES buffer, 10 mM, pH 6.5).

distributed between C_{16}- and C_{18}-acids (Fig. 2 A + B). Only the unsaturated C_{16}-acids bound to 2-MGMG and MGDG (Fig. 2 C) reflect approximately the opposing ^{14}C-incorporation behavior observed previously for the fatty acid residues of these lipids [3, 4]. This observation thus corroborates conclusions from the preceding experiment. The possible importance of long-chain thioesters (acyl-ACP or acyl-CoA) for the selective introduction of fatty acids into the glycerol residue of galactolipids, has been mentioned above [5, 6].

To investigate the thioester requirement during monoglyceride accumulation, chloroplast extract (stroma) and envelope fractions of a defined protein concentration (isolated from spinach chloroplasts according to Joyard and Douce [9]) were combined and incubated with sn-[^{14}C]-glycerol-3-phosphate in the presence of various acyl donors at pH 8.0. Alkaline conditions were chosen in order to optimize fatty acid synthesis in the stroma [9] as well as the galactolipid synthesis in the envelope membranes [17]. Envelope membranes were added to introduce the secondary acylation as well as the galactosylation (in the presence of UDP-galactose) of the glycerol backbone in chloroplast lipids [9]. After 2 h incubation allowing for a sufficient accumulation of glyceride-precursor, galactolipid synthesis was then induced by addition of UDP-galactose.

The data of Table II clearly show that oleoyl-CoA is an effective acyl donor for glyceride formation which in turn starts the galactosylation process after short incubation periods (compare the control value), while palmitoyl-CoA is ineffective. However, a combination of both is highly effective in stimulating the galactose addition to the glyceride after prolonged incubation periods. This finding supports the fatty acid selectivity of the above mentioned acyltransferases, claimed by other authors [5, 16], which catalyzes the successive esterification of sn-glycerol-3-phosphate first by C_{18}- and afterwards by C_{16}-acids if only long-chain acyl-CoA molecules are offered as acyl donors. As expected, the label in the MGDG fraction increases while that of the DG fraction decreases [9]. On the other hand, the relatively poor and stabel labeling of the occurring monoglycerides and monogalactosyl monoglycerides (Table II) is not likely to demonstrate the expected galactosylation of monoglycerides (see Scheme 1). This observation suggests, that the accumulation of specific monoglyceride species (Table I, Fig. 2C) is required for further galactosylation.

As shown by the preceding experiments the acylation mechanism of sn-glycerol-3-phosphate is dependent on the supply of specific long-chain fatty acids. The latter is influenced by substrate and cofactor concentrations [16, 18], by temperature [19] and, as shown above, by the pH of the incubation medium (Table I). For a systematic investigation of this pH influence the fatty acid turnover in long-chain acyl-ACP-compounds during lipid synthesis in a partially reconstituted chloroplast system, which consisted of a stroma-envelope combination was studied. In contrast to the preceding experiment, the stroma phase containing fatty acid synthetase [20] was successively incubated first with [1-^{14}C]acetate (90 min) at pH 8.9 under optimal conditions required for fatty acid synthesis [9] secondly with sn-glycerol-3-phosphate (30 min) after adjustment of the pH (pH 6.5 or 8.9) to introduce lysophosphatidic and monoglyceride synthesis in the stroma, and

Table II. Lipid synthesis by a combination of chloroplast stroma- and envelope-fractions measured by the incorporation of sn-glycerol-3-phosphate at pH 8.0 in the presence of a) 0.5 mM CoA and 4 mM ATP; b) 0.1 mM oleoyl-CoA; c) 0.1 mM palmitoyl-CoA; d) 0.1 mM palmitoyl-CoA and 0.1 mM oleoyl-CoA. Beside the cofactors (a – d) the incubation medium contained: Tricine/NaOH buffer, 10 mM, pH 8.0; sucrose, 0.33 M; $MgCl_2$, 1 mM; sn-glycerol-3-phosphate, 1 mM; 4 μCi sn-[^{14}C]glycerol-3-phosphate (specific activity 171 mCi/mmol); stroma proteins 2,1 mg/ml and envelope proteins 0.16 mg/ml. After 2 h of incubation galactolipid synthesis was started by the addition of UDP-galactose (0.5 mM).

Lipid		sn-[^{14}C]glycerol-3-phosphate incorporated into lipids [nmol × mg^{-1} proteins] (time after addition of UDP-gal. [min])					
		0	5	15	20	30	60
MGDG	a) (+ CoA, + ATP)	0.23	0.38		0.74		2.16
	b) (+ $C_{18:1}$-CoA)	0.50	1.17	1.57	1.92		1.99
	c) (+ $C_{16:0}$-CoA)	0.03	0.02	0.02		0.02	
	d) (+ $C_{16:0/18:1}$-CoA)	0.49	1.02		1.99		3.31
DG	a)	0.94	0.70		0.86		1.74
	b)	2.52	1.86	1.90	2.03	1.44	1.27
	c)	0.06	0.03	0.03		0.02	
	d)	2.89	2.12		2.82		3.89
1-MG	a)	0.05	0.08		0.06		0.19
+ 2-MG	b)	0.07	0.12		0.08		0.09
	c)*						
	d)	0.08	0.10		0.19		0.34
1-MGMG	a)	0.06	0.07		0.05		0.09
+ 2-MGMG	b)	0.06	0.07	0.11	0.04		0.04
	c)*						
	d)	0.07	0.08		0.07		0.13

* Only traces of radioactivity were found.

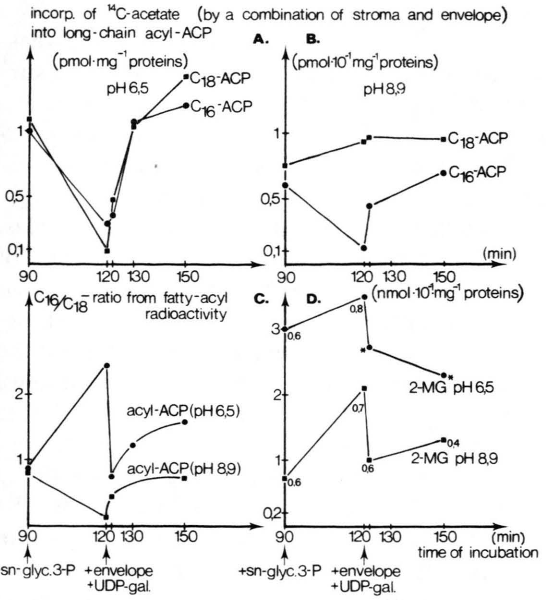

Fig. 3. Effect of the pH on the distribution of radioactivity between C_{16}- and C_{18}- fatty acids (expressed as $^{14}C_{16}/^{14}C_{18}$-ratio; C) bound in long-chain acyl-ACP (A, B) during lipid synthesis from [1-^{14}C]acetate by a mixture of chloroplast stroma and envelopes. D. shows the simultaneous ^{14}C-incorporation kinetics into 2-monoglycerides (2-MG) while the $^{14}C_{16}/^{14}C_{18}$-ratio in 2-MG is given by the numbers under this curves (*not estimated). Radioactivity of all fatty acids with the same chain length was summarized. Stroma fractions equivalent to 9.9. mg (A) and 4.5 mg (B) proteins were incubated for 90 min at 25 ° in 1.5 ml of a basal medium containing: Tricine/NaOH buffer, 10 mM, pH 8.9; CoA, 0.1 mM; ATP, 4 mM; NADPH, 0.5 mM; NADH, 0.5 mM; $NaHCO_3$, 1 mM; DTT, 1 mM; [1-^{14}C]acetate, 1.76 mM (specific activity: 58.2 mCi × mmol^{-1}). After adjustment of the pH to 6.5 (A) or 8.9 (B), lipid synthesis was continued

1st by addition of sn-glycerol-3-phosphate (2 mM) after 90 min.

2nd by addition of envelope membranes (= 0.8 mg proteins) and UDP-galactose (0.5 mM) after 120 min.

thirdly with envelopes and UDP-galactose for further diglyceride and galactolipid synthesis.

Fig. 3 shows the results: Addition of sn-glycerol-3-phosphate to the pure stroma fraction leads to a decrease of radioactivity in ACP-bound long-chain fatty acids (Fig. 3A, B; except for C_{18}-acids at pH 8.9). The simultanous increase of label in the 2-monoglyceride fraction indicates a direct fatty acid transfer from acyl-ACP into the C-2-position of sn-glycerol-3-phosphate by the "soluble" acyltransferase in the chloroplast stroma, because the usually preceding transesterification to acyl-CoA (acyl-CoA-synthetase), preferentially transfering fatty acids into the C-1-position (Fig. 1, Tables I and II), is likely bound to the envelope membrane [9]. In order to analyse the fatty acid turnover by long-chain acyl-ACP during sn-glycerol-3-phosphate acylation at different pH, radioactivity (from [1-^{14}C]acetate) in the thioester-bound C_{16}-and C_{18}-fatty acids was compared and is represented as $^{14}C_{16}/^{14}C_{18}$-ratio in Fig. 3C. This comparison indicates an ACP-bound accumulation of C_{16}-fatty acids at pH 6.5 or of C_{18}-acids at pH 8.9 after addition of sn-glycerol-3-phosphate to the stroma fraction. This observation suggests, that pH differences in the chloroplast stroma during the acylation of sn-glycerol-3-phosphate bring about changes in ACP- bound C_{16}- and C_{18}-fatty acids, and are thus responsible for the different fatty acid incorporation into monoacyllipids under both pH-conditions (Table I). Independent of the fatty acid species released from ACP under different pH conditions (Fig. 3C), the incorporation of C_{16}-fatty acids into 2-monoglycerides seems to predominate (see C_{16}/C_{18}-ratio, given by the numbers under the ^{14}C-incorporation kinetics of Fig. 3D). After addition of envelope membranes and UDP-galactose a decrease of label in the 2-monoglyceride fraction (Fig. 3D) as well as a rapid new synthesis of long-chain acyl-ACP (Fig. 3A) is observed. Both observations point to the further metabolization of those lipid intermediates

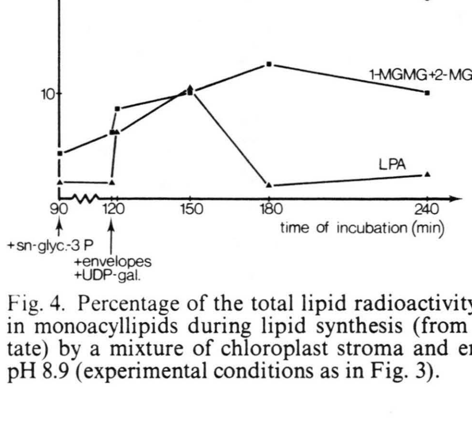

Fig. 4. Percentage of the total lipid radioactivity measured in monoacyllipids during lipid synthesis (from [1-^{14}C]acetate) by a mixture of chloroplast stroma and envelopes at pH 8.9 (experimental conditions as in Fig. 3).

(2-MG for example), accumulating in the pure stroma fraction prior to envelope addition. Fig. 4 additionally illustrates the relative decline of 2-MG, accumulated by acylation of sn-glycerol-3-phosphate at pH 8.9 in a pure stroma fraction, when envelope membranes and UDP-galactose are present. This decline of 2-MG, predominantly involving the C_{16}-species, seems to be compensated by the simultaneous increase of other chloroplast lipids, two species of which (lysophosphatidic acids and monogalactosyl monoglycerides) are depicted in Fig. 4. The apparent synthesis of monogalactosyl monoglycerides (1- and 2-MGMG) at the expense of monoglycerides indicates the expected galactosylation of monoglycerides under defined conditions represented in Scheme 1.

Discussion

During diglyceride synthesis in chloroplasts the successive acylation of sn-glycerol-3-phosphate is catalyzed by two acyl transferases. While the esterification of the first fatty acid leading to LPA-formation is found in the stroma fraction, the further acylation to phosphatidic acid is strictly bound to the envelope membrane [9, 20]. Probably because of the rapid turnover of the intermediary LPA, their direct demonstration in intact chloroplasts failed as yet [6, 18]. Only monoglycerides, formed by the dephosphorylation of LPA [9], as well as their galactosylated derivatives [4] were found in trace amounts in lipid extracts of intact chloroplasts. The fatty acid turnover in these monoacyllipids, however, provides an indicator for the simultaneous primary acylation of sn-glycerol-3-phosphate. This is supported by the observation that we found, in most of our experiments, two monoglyceride-(1-MG and 2-MG) and monogalactosyl monoglyceride isomers (1-MGMG and 2-MGMG) [4] with small differences in their polarity. While the occurrence of 1- and 2-monoglycerides was clearly demonstrated (see Methods), the lower polarity monogalactosylmonoglyceride isomer, previously called 1-MGMG [4], was designated as 2-MGMG, although the direct proof of the 2-O-acyl-bond failed as yet. This is presumably due to the generally complicated preparative procedures for isolation and analysis of the 2-O-acyl-3-O-β-D-galactopyranosyl-sn-glycerol (2-MGMG) [21]. Starting with the question on the origin of monogalactosyl monoglycerides, a significant accumulation of these lipids [4] together with monoglycerides was achieved in cell free systems, especially under weakly acidic conditions (Table I, Fig. 1). From the simultaneous occurrence of both monoacyllipids the formation of monogalactosyl monoglycerides by galactosylation of monoglycerides is indicated by the experiments in Table I and Fig. 4. The accumulation of monoglycerides may be due to a loss of chloroplast integrity, presumably leading to an interruption of the concerted action of both acyltransferases, bound to different chloroplast compartments [20]. An accumulation of 2-monoglycerides as indicators for the primary acylation of the C-2-position of the glycerol residue in plastidary lipids was observed only during glyceride synthesis by [1-^{14}C]acetate-labeled chloroplast extract (stroma) fractions (Fig. 3), containing long-chain acyl-ACP but no acyl-CoA [9, 22]. The presence of envelope membranes (*i. e.* of long-chain acyl-CoA; Fig. 1, Table II) led to a predominant formation of 1-monoglycerides. Therefore the positional specificity of the primary acylation of sn-glycerol-3-phosphate seems to be determined by the nature of the acyl donor supplied (acyl-ACP or acyl-CoA) and to be independent of the chain length of the fatty acid transferred. This conclusion is indicated by the simultaneous occurrence of C_{16}- and C_{18}- species of both monoglyceride isomers (Figs. 3 and 4).

The accumulation of 1-monoglycerides together with the apolar monogalactosyl monoglyceride isomer (2-MGMG) in gently shocked chloroplast suspensions under acidic conditions (Table I) suggests a preferential galactosylation of 2-monoglycerides, probably because of the position or the nature of the fatty acids bound to these lipids. This finding is supported by the stimulation of the interlipid-galactosyltransferase activity, measured in envelope membranes under acidic conditions [17]. Simultaneously with the accumulation of monoacyllipids under acidic conditions the incorporation of C_{16}-fatty acids, especially unsaturated ones, into these lipids was significantly increased (Table I). The 2-monoglyceride bound C_{16}-fatty acids appear to originate directly from long-chain acyl-ACP, where they accumulate under acidic conditions (Fig. 3). Because of the rapid elongation of ACP-bound C_{16}-fatty acids under normal conditions [23], their accumulation may be due to an inhibition of the elongase system. On the other hand, palmitoyl-CoA proved a poor acyl-donor for the primary acylation of sn-glycerol-3-phosphate if compared to oleoyl-CoA (Table II). Consequently, if C_{16}-fatty acids are first ligated to the C_2-position of sn-glycerol-3-phosphate as suggested by other authors [6, 18] as well as by our own experiments (Table I, Fig. 2), this positional specificity of the acyltransferase should be due to the direct use of acyl-ACP substrates as acyl-donor [24]. Factors leading to an accumulation of ACP-bound C_{16}-fatty acids, like temperature [19], sn-glycerol-3-phosphate concentration [18] and the pH, may influence the directed introduction of C_{16}-acids into the glycerol residue of chloroplast lipids. That these observations may also hold for *in vivo* conditions is supported by an accumulation of hexadecatrienoic acid ($C_{16:3}$) in 2-MGMG of spinach leaves under certain conditions [3] as well as by the incorporation kinetics of [1-^{14}C]acetate into monoglycerides and galactolipids of the liverwort *Ricciocarpos natans* [25].

Acknowledgements

The investigation was supported by a grant from the Deutsche Forschungsgemeinschaft.

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