Similarities and Differences in Lipid Metabolism of Chloroplasts Isolated from 18:3 and 16:3 Plants

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ABSTRACT

Photosynthetically active chloroplasts retaining high rates of fatty acid synthesis from [1-14C]acetate were purified from leaves of both 16:3 (Solanum nodiflorum, Chenopodium album) and 18:3 plants (Amaranthus lividus, Pism sativum). A comparison of lipids into which newly synthesized fatty acids were incorppated revealed that, in 18:3 chloroplasts, enzymic activities catalyzing the conversion of phosphatidate to diacylglycerol and of diacylglycerol to monogalactosyldiacylglycerol (MGD) were significantly less active than in 16:3 chloroplasts. In contrast, labeling rates of MGD from UDP-14Cglycerol were similar for both types of chloroplasts.

The composition and positional distribution of labeled fatty acids within the glycerides synthesized by isolated 16:3 and 18:3 chloroplasts were similar and in each case only a C18/C16 diacylglycerol backbone was synthesized. In nodiflorum chloroplasts, C18/1/C16 MGD assembled de novo was completely desaturated to the C18:3/C16:3 stage.

Whereas newly synthesized C18/C18 MGD could not be detected in any of these chloroplasts if incubated with [14C]acetate after isolation, chloroplasts isolated from acetate-labeled leaves contained MGD with labeled C18 fatty acids at both sn-1 and sn-2 positions. Taken together, these results provide further evidence on an organellar level for the operation of pro- and eucaryotic pathways in the biosynthesis of MGD in different groups of plants.

A major unsolved problem in plant lipid biochemistry is the biosynthesis of trienoic acids such as linolenic and hexadecatrienoic acid. The sequential introduction of double bonds into saturated acyl chains may involve different substrates and compartments in different plants and organs. Plastids always play a very important role in these reactions, the essential points of which may be summarized as follows (21).

In leaves of 18:3 plants, chloroplasts synthesize stearoyl-ACP 2 in the stroma, introduce the first double bond into the saturated hydrocarbon chain, and then hydrolyze the thioester (23). Released oleate is exported across chloroplast envelopes into membranes of the eucaryotic part of the cell, probably the ER, where it is incorporated into PC. PC-linked oleoyl groups are desaturated in these membranes (27) and subsequently move back into the chloroplast. In the envelope (3), galactosylation of imported DAG having two linoleoyl groups results in an accordingly unsaturated MGD. The MGD-linked acyl groups are substrates for the introduction of the third double bond (16) to yield MGD with two linolenyl residues. This galactolipid is characteristic of 18:3 plants such as Asteraceae and Fabaceae, for example (9). It is called eucaryotic MGD, because the nucleocytoplasmic part of the cell controls the construction of the DAG backbone which carries C18 fatty acids at both C-1 and C-2 positions of glycerol (7), whereas C16:0, if present, is excluded from C-2.

In photosynthetically active cells of 16:3 plants which are represented, for example, by members of Apiaceae and Brassicaceae (9), two pathways operate in parallel (18) to provide thylakoids with MGD. The cooperative 'eucaryotic' sequence (4) is supplemented to various extents by a 'procytotic' pathway. Its reactions are confined to the chloroplast and result in a typical arrangement of acyl groups as well as their complete desaturation once they are esterified to MGD. Procytotic DAG backbones carry C16:0 and its desaturation products at C-2 (7) from which position C18 fatty acids are excluded. The C-1 position is occupied by C18 fatty acids and to a small extent by C16 groups. The similarity in DAG backbones of lipids from blue-green algae (22, 30) with those synthesized by the chloroplast-confined pathway in 16:3 plants suggests a phylogenetic relation and justifies the term procytotic.

The different pathways just mentioned were deduced from labeling experiments with leaves from a small selection of 18:3 and 16:3 plants (17, 24, 26, 29), whereas studies with isolated chloroplasts were mainly confined to organelles from the 16:3 plant spinach (14, 20). To provide additional evidence for the roles ascribed to chloroplasts in pro- or eucaryotic pathways during MGD biosynthesis in different plants, we carried out experiments with chloroplasts isolated from 18:3 as well as 16:3 plants. Our data suggest that chloroplasts from these two groups of plants have similarities and differences in enzymic equipment explaining some of the above mentioned complications in lipid biosynthesis.

MATERIALS AND METHODS

Plants. Pism sativum L. cv Victory Freezer was grown from seed for 10 to 20 d in the greenhouse. Amaranthus lividus L. (amaranth), Chenopodium album L. (fat hen), and Solanum nodiflorum L. (nightshade) were collected as weeds in the fields.

Chloroplast Isolation. The medium (12) used for isolating chloroplasts, forming gradients, measuring O2 evolution and lipid synthesis contained 25 mM Hepes, 10 mM KH2PO4, 2 mM EDTA, 1 mM MgCl2, 1 mM MnCl2, 0.3 mM KH2PO4, and 0.33 M sorbitol, adjusted to pH 7.9. Linear Percoll gradients were mixed from 17 ml of commercial Percoll suspension containing 0.33 M sorbitol and 17 ml of isolation medium.

About 50 g of leaves were quickly cut into small pieces with a razor blade, placed into semiconfined medium (150 ml), and homogenized for 3 to 5 s with a Polytron. The homogenate was filtered through two layers of Miracloth into four centrifuge tubes

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2 Abbreviations: ACP, acyl carrier protein; DAG, diacylglycerol; LPA, lysophosphatidic acid; MGD, DGD, and SQD, monogalactosyldigalactosyldiacylglycerol; PA, phosphatidic acid; PC and PG, phosphatidylycerrine and -glycerol; UFA, unesterified fatty acids.
Unmodified and hydrogenated (1) fatty acid methyl esters were also separated by reverse-phase TLC on paraffin-impregnated silica gel G developed with acetonitrile:methanol:H₂O (6:3:1, v/v). Positional distribution of fatty acids was analyzed with lipase from Rhizopus (24). For a complete analysis of weakly labeled intermediates, several parallel incubations had to be combined.

**RESULTS AND DISCUSSION**

**Isolation of Chloroplasts.** Following attempts to prepare intact and biosynthetically active chloroplasts from a number of species, we selected two 183 (Pisum, Amaranthus) and two 163 plants (Chenopodium, Solanum) to study lipid synthesis by the different types of chloroplasts. Chloroplasts were obtained in pure form after centrifugation on a Percoll gradient within 15 min. The resulting organelles gave single bands upon recentrifugation on Percoll or sucrose gradients. Accordingly, we found high ratios of total as well as soluble protein per Chl (Table I) in agreement with previous results (12). The low content of soluble protein in *Amaranthus* chloroplasts may be explained by the absence of carboxydismutase from these organelles (13) which represent the mesophyll chloroplasts of a C4 plant. This resulted in their separation from the other chloroplasts listed in Table I during centrifugation in sucrose or Percoll gradients in which they were banding just above the chloroplasts from the C3 plants. *Amaranthus* chloroplasts, in contrast to the others listed in Table I, did not show CO₂-dependent O₂ evolution. In agreement with previous results (10), they evolved O₂ on addition of 3-P glycerate, oxaloacetate, or nitrite. Oxaloacetate-dependent rates were approximately doubled by addition of pyruvate or NH₄Cl.

**Acetate Incorporation.** The chloroplasts isolated from the four different plants incorporated acetate into fatty acids under conditions which had been optimized for spinach (18). The maximum rates of about 1 μmol/mg Chl-h are lower than those of spinach for which rates in excess of 2 μmol/mg Chl-h have been measured (25). But they demonstrate that the capacity to synthesize fatty acids from acetate is developed to a similar degree in other chloroplasts as well. It should be mentioned that chloroplasts with acetate-incorporating activities as high as those listed in Table I have been prepared so far only from spinach leaves. 183 and 163 chloroplasts apparently do not differ with respect to their capacity to synthesize fatty acids from acetate which is considered to be the physiological substrate in spinach leaves where its concentration may reach 1 mM (11).

**Table I. Some Characteristics of Intact Chloroplasts Isolated from Leaves of Solanum nodiflorum, Chenopodium album, Amaranthus lividus, and Pisum sativum**

<table>
<thead>
<tr>
<th>Species</th>
<th>Total protein, mg/mg Chl</th>
<th>Soluble protein, mg/mg Chl</th>
<th>O₂ evolution, μmol/mg Chl-h</th>
<th>Acetate incorporation, nmol/mg Chl-h</th>
<th>MGD labeling, nmol/mg Chl-h</th>
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<tr>
<td>Solanum</td>
<td>31</td>
<td>17</td>
<td>63</td>
<td>791</td>
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<tr>
<td>Chenopodium</td>
<td>19</td>
<td>20</td>
<td>96</td>
<td>1647</td>
<td></td>
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<td>Amaranthus</td>
<td>14</td>
<td>5</td>
<td>130</td>
<td>855</td>
<td></td>
</tr>
<tr>
<td>Pisum</td>
<td>24</td>
<td>14</td>
<td>42</td>
<td>1086</td>
<td></td>
</tr>
</tbody>
</table>

The first two species are 163, the last two are 183 plants. Most data are means from several independent preparations: two to three for proteins, five to seven for O₂ evolution (CO₂-dependent rates for all but *A. lividus* which is a 3-P glycerate-dependent rate), and two to four for labeling of MGD (under conditions of the MGD mode in the presence of UDP-[1-14]C)gyl. For acetate incorporation, only maximal figures are given, whereas means from three to six independent preparations reached 43 to 77% of these rates.

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Substrate mixtures and incubation conditions have been worked out for spinach chloroplasts (18) which allow the flow of newly synthesized fatty acids to be directed into several different products. Similar results were obtained with the different chloroplast preparations described above suggesting a general applicability of these mixtures. For the present discussion, we describe the experiments carried out with two different incubation mixtures. The essential substrates were [1-¹⁴C]acetate and glycerol 3-P (DAG mode) or the same mixture with additional UDP-gal (MGD mode). Apart from UFA, which were always present in appreciable proportions, DAG and MGD, respectively, predominated as reaction products. Rates of fatty acid synthesis (Table I) were determined from acetate incorporated into lipids during a 15-min incubation in the light. During an additional 60-min incubation in the dark, acetate is not further incorporated, but labeled lipids were metabolized further, particularly regarding reactions of glyceride synthesis and acyl group desaturation. The labeling of the predominant reaction products is shown in Figure 1 together with previously obtained data for spinach chloroplasts (18).

**Lipid Labeling in the DAG Mode.** When chloroplasts were incubated with [¹⁴C]acetate in the DAG mode (Fig. 1, left), all five plants incorporated a high proportion of label into UFA. This percentage was higher in 18:3 (amaranth, pea) as compared to 16:3 chloroplasts. Compared with spinach and *Solanum, Chenopodium* directed a larger proportion of acetate into UFA which supports its position between extreme 16:3 and 18:3 plants. The elevated production of UFA in 18:3 chloroplasts could be related to a difference in the formation of PA. The rate of PA synthesis, which is calculated by combining the radioactivities in PA and DAG, decreased from 16:3 to 18:3 plants by a factor of two. In addition, the hydrolysis of PA as indicated by the label in DAG decreases by a factor of four when comparing 16:3 with 18:3 chloroplasts in which a large proportion of PA was not hydrolyzed to DAG even after 75 min. The small proportion of DAG cannot be due to a limited supply of PA but suggests a reduced activity of PA phosphatase which catalyzes this interconversion in chloroplast envelopes (3). This is supported by two recent observations: isolated envelopes from pea and spinach chloroplasts showed pronounced differences in the activity of PA phosphatase (5), and the turnover rate of PA was 25-fold greater in chloroplasts from 16:3 compared with 18:3 plants (S. E. Gardiner and P. G. Roughan, in preparation).

To summarize, the incubations in the DAG mode showed that 18:3 chloroplasts direct less fatty acids into PA and DAG synthesis than did 16:3 chloroplasts despite the fact that both types have similar capacities for fatty acid synthesis. This reduction in glyceride synthesis suggests a reduced capacity for PA synthesis and an even more reduced activity of PA phosphatase.

**Lipid Labeling in the MGD Mode.** Before discussing the MGD biosynthesis, it should be mentioned that incubations in the DAG or MGD mode did not result in significant acetate incorporation into lipids such as DGD, SQD, PC, or PG, which individually contained only 0.1 to 1% of the total lipid radioactivity. The low labeling of PC and PG differs from results which were obtained by other authors after incubating chloroplasts under slightly different conditions. The absence of label in PC (4) may be ascribed to the absence of CoA and ATP in our incubations. These substrates are necessary for acyl-CoA formation at the outside of chloroplasts (19) and its subsequent incorporation into chloroplast PC (2). PG was labeled in chloroplasts (15) only in the absence of Triton X-100, whereas 0.008% of this detergent was used in our incubations.

Without addition of UDP-gal, hardly any conversion of DAG to MGD was observed in agreement with the extraplasmatic location of the synthesis of this sugar nucleotide (7). In the presence of UDP-gal (Fig. 1, right), high rates of MGD formation were seen in 16:3 chloroplasts (*Spinacia, Solanum*), whereas *Chenopodium* had an intermediate position. In contrast, 18:3 chloroplasts (*Pisum, Amaranthus*) synthesized MGD at rates which were only about 5% of those observed in 16:3 chloroplasts. This difference was particularly evident in the 15-min incubations. Although 18:3 chloroplasts synthesized some DAG, most of it could not be converted to MGD. Therefore, apart from low absolute labeling of MGD, 18:3 chloroplasts differed from 16:3 organelles also in low ratios for MGD/DAG labeling. The presence of residual DAG at the end of these incubations indicated that not only a reduction in the synthesis of the galactose acceptor but also a decrease in its conversion to MGD contributed significantly to the deficiency in MGD synthesis by 18:3 chloroplasts. This suggests that another difference between 16:3 and 18:3 chloroplasts is the proportion of newly synthesized DAG which can be converted to MGD. Whether this is due to a reduced activity of a particular galactosyl transferase or to a limited accessibility of newly synthesized DAG to an active galactosyltransferase cannot be decided at present. Therefore, these observations are not necessarily in contrast to the results in Table I which showed that galactosylation rates measured with 16:3 and 18:3 chloroplasts were similar. The
data in Table I are based on the galactosylation of preexisting and highly unsaturated DAG (2), whereas those in Figure 1 depend on the conversion of newly synthesized DAG. Another explanation of the differences in galactosylation rates in Table I and Figure 1 would be the existence of two different galactosylating activities, a procaryotic enzyme involved in the conversion of newly synthesized DAG as part of a concerted process leading to fully desaturated MGD, and a eucaryotic activity responsible for the galactosylation of preexisting or imported DAG of eucaryotic structure. Further studies on an enzymic level are required to clarify these questions.

Despite this presently unresolved discrepancy, the incubations in the MGD mode demonstrate that 18:3 chloroplasts compared to 16:3 organelles synthesize far less MGD, because they produce less DAG and convert less of this to MGD. It is evident that these differences between 18:3 and 16:3 chloroplasts are in line with the differences between eu- and procaryotic pathways of MGD biosynthesis in 18:3 and 16:3 plants as outlined above.

Labeled Fatty Acids in Individual Lipids. An important question regarding the introduction of double bonds into acyl groups is the nature of the acyl-carrying substrate. As mentioned above, C18:1 is formed at the level of an ACP-thioester, whereas the actual substrates for the introduction of the second and third double bonds seem to be C18:1 groups in PC and MGD. In the case of C16:0, even the first double bond seems to be introduced only when these residues are esterified in lipids such as PG and MGD (21). Therefore, we compared the acyl groups labeled in intermediates up to the stage of DAG, which are assumed to have no function in polyunsaturation, with those in MGD which is a substrate for further desaturation.

Separating fatty acids according to number of double bonds by argentation TLC does not differentiate according to chain length. Therefore, the fatty acids in Figure 2 are characterized only by number of double bonds, whereas in the text we refer to monoenoic acids as being mainly C18:1 and to saturated acids as being mainly C16:0. Reversed-phase TLC of fatty acid methyl esters from various MGD samples (see below) justified this simplification.

UFA and LPA (Fig. 2) had very similar fatty acid profiles with a predominance of C18:1. UFA are not the immediate products of fatty acid biosynthesis. Their release from an ACP-thioester pool in which C16:0 and C18:1 predominate (28) is controlled by the specificity of an ACP-thioesterase (23). The purified enzyme showed highest activity towards C18:1-ACP which is in agreement with the acyl composition of UFA (Fig. 2).

The similar predominance of C18:1 in LPA reflects the selectivity of the stroma enzyme glycerol 3-P acyltransferase which has recently been characterized from a 16:3 as well as a 18:3 plant (5). Regardless of its origin, this enzyme prefer C18:1-ACP from a mixture of acyl-ACP to acylate the C-1 position of glycerol 3-P.

In PA, DAG, and MGD, an increased proportion of C16:0 is observed in both 16:3 and 18:3 plants. The enrichment of C16:0 in these lipids as compared to LPA is in agreement with the selectivity of the acylglycerol 3-P acyltransferase from chloroplast envelopes of both 16:3 and 18:3 plants (5). This enzyme prefers C16:0-ACP for the acylation of the C-2 position in LPA. In most samples, C16:0 was present in excess of 50% and, accordingly, separation of molecular species of PA, DAG, and MGD by argentation TLC showed the presence of some fully saturated species running ahead of the predominating C18:1/C16:0 combination.

The MGD samples analyzed for Figure 2 were obtained from 15-min assays, since longer incubation times may result in desaturation of acyl groups. But even after 15 min, small proportions of dienoic acids were already present in MGD, whereas all other compounds, whether analyzed after 15 or 75 min, contained only insignificantly low proportions of polyenoic acids, thus confirming the roles ascribed to the various lipids and intermediates. MGD of a similar fatty acid composition as shown in Figure 2 for peas was recently found to be synthesized in an independent study of pea chloroplasts (4) and by the combined action of the two acyltransferases in a reconstituted system from pea chloroplasts (5). Isolated spinach chloroplasts produced similar profiles of labeled fatty acids in various intermediates (14, 18, 20).

In summary, the analysis of fatty acid composition does not reveal differences with regard to the fatty acids formed or incorporated during short-term incubations into lipids by 16:3 and 18:3 chloroplasts, since all DAG-containing lipids have about equal proportions of saturated and monoenoic acids.

Positional Distribution of Fatty Acids. A sensitive criterion for differentiation between pro- and eucaryotic DAG backbones is the distribution of fatty acids between the C-1 and C-2 position. Particularly useful is the fatty acid mixture at C-2, since the ratio of C16- to C18-acyl chains reflects the contribution of pro- and eucaryotic pathways (21). The positional distribution of labeled acyl groups in PA and MGD are shown in Figure 3.
of acyl groups in PA and DAG synthesized by isolated chloroplasts. In each case, only a prokaryotic type of DAG backbone was synthesized with C18:1 and a small proportion of C16:0 at C-1 and C16:0 at C-2. The same pattern shows up in MGD but in this lipid individual fatty acids may be transformed by subsequent introduction of double bonds.

**Desaturation of Prokaryotic MGD.** Prior to the present study, the ability of chloroplasts to desaturate MGD-bound acyl-chains has been demonstrated only with spinach chloroplasts (20). Identical experiments with chloroplasts from *Solanum* and *Chenopodium* confirmed the autonomy of these organelles in the production of trienoic acyl chains as components of de novo-synthesized MGD. As already seen from Figures 2 and 3, *Solanum* chloroplasts showed the highest activity in desaturating MGD. These results, obtained by argentation TLC of total fatty acids, were confirmed by resolving molecular species of intact MGD and by separating fatty acids from C-1 and C-2 by reversed-phase TLC before and after hydrogenation. This technique shows the extent of desaturation at both positions as well as the purity with regard to the prokaryotic structure of the DAG backbone.

The absence of C18 fatty acids from the C-2 position of *Solanum* MGD as evident after hydrogenation and reversed-phase TLC (Fig. 4) demonstrates that the MGD synthesized by these chloroplasts has a purely prokaryotic DAG backbone. Before desaturation, it carried C18:1 at C-1 and C16:0 at C-2 as shown above (Fig. 3). In combination with previous studies on the time-dependent changes of fatty acids from in vivo-labeled MGD (24), these data show that the desaturation sequence at the C-2 position starts with a fully saturated acyl substrate and leads via C16:1 and C16:2 to C16:3 by introducing three double bonds (Fig. 4). At the C-1 position, only two double bonds are introduced into C18:1 to reach the trienoic level. MGD synthesis is prerequisite for 18:1 and 16:0 desaturation. This strongly suggests, but does not prove, desaturation of MGD-bound acyl groups. Since desaturation continued in the dark, light is apparently not required for desaturation reactions. On the other hand, some cofactors synthesized in the light, may still have been available in sufficient quantities in the subsequent dark incubation. Whether or not identical enzymes introduce the second and third double bonds into C-1 and C-2 acyl groups of prokaryotic MGD cannot be answered at present.

The observation that desaturation of *Chenopodium* MGD was confined to the C-1 position (Fig. 3) may indicate the existence of a separate group of enzymes for the C-2 position. Even a third group could be responsible for the desaturation of C18 chains at C-2 of eucaryotic MGD. We also cannot answer the question whether or not C16:0 at C-1 of prokaryotic MGD can be desaturated. If this reaction does not occur (see Fig. 3), then the desaturation of C16:0 is specifically linked to C-2.

The resolution of *Solanum* MGD into molecular species (Fig. 5) reveals a complete series of labeled intermediates filling the gap between C18:1/C16:0 and C18:3/C16:3 combinations. The absence of label in the position of the eucaryotic C18:3/C18:3 species is very significant. This, together with the prokaryotic C18:3/C16:3 combination predominate the spectrum of unlaabeled species as detected by dichlorofluoresceine spraying. For comparison, the molecular species of pea MGD are also shown to demonstrate the poor desaturation in these chloroplasts, although they were highly active in O2 evolution and acetate incorporation. A very similar pattern was observed with *Amaranthus* MGD. Whether the poor desaturation of prokaryotic MGD in 18:3 chloroplasts is a further characteristic of these organelles due to the absence of specific desaturases or whether it is a consequence of inadequate conditions during isolation and incubation cannot be answered at present.

The experiments with spinach (20) and *Solanum* suggest that 16:3 chloroplasts desaturate both acyl chains of de novo-synthesized C18:1/C16:0 MGD in a light-independent reaction to yield

![Figure 3](https://i.imgur.com/3Y5Z5Z5.png)

**Fig. 3.** Positional distribution of labeled fatty acids between C-1 and C-2 positions of PA and MGD synthesized by isolated chloroplasts during incubations for 75 min. All samples were subjected to hydrolysis with lipase from *Rhizopus*. In each compound, percentages at both positions together sum up to 100%. Abbreviations of plants as in Figure 2.

PA samples, obtained from 75-min incubations, did not contain significant proportions of polyenoic acids. C16:0 and C18:1 are distributed between C-1 and C-2 in agreement with the specificity of the chloroplast acyltransferases which are involved in the synthesis of this intermediate (5). The patterns at C-1 reflect LPA patterns (Fig. 2) and showed the preference of C18:1 as well as a small allowance for C16:0. The C-2 position is occupied rather exclusively by C16:0 in agreement with the higher selectivity of the second acyltransferase (5). It is evident that this is a purely prokaryotic arrangement.

The MGD samples analyzed for Figure 3 were obtained from incubations lasting for 75 min instead of 15 min as used for Figure 2. Nevertheless, the same fatty acids and the same positional distribution as observed in PA were found in MGD samples from pea and amaranth, whereas in *Chenopodium* only the fatty acids at C-2 were the same in both lipids. At C-1, C18:2 and C18:3 occurred in addition to C16:0 and C18:1. This is ascribed to desaturation at the C-1 position of MGD which had deformed the originally simple pattern. Even though further desaturation at both positions of *Solanum* MGD had produced a complex pattern, it had the same prokaryotic pattern as shown by reversed-phase TLC (see below), since no C18 chains were found at C-2. Previous studies with isolated spinach chloroplasts (14, 18, 20) resulted in the same distributions as those shown in Figure 3.

Therefore, there were no significant differences between 16:3 and 18:3 plants regarding composition and positional distribution.
FIG. 4. Desaturation of procaryotic MGD by isolated chloroplasts as shown by analysis of constituent fatty acids. Chloroplasts from Solanum nodiflorum were incubated in the MGD mode for 75 min for subsequent isolation of acetate-labeled MGD. The fatty acid mixtures esterified at the C-1 and C-2 position were obtained by lipase hydrolysis and analyzed by reversed-phase TLC in acetonitrile:methanol:1H2O (6:3:1, v/v) followed by radioautography. TLC of hydrogenated samples confirms the assignments of individual fatty acids which could otherwise have comigrated as critical pairs, e.g. C16:0 and C18:1. The absence of C18:0 at C-2 after hydrogenation is evidence for a procaryotic DAG backbone.

FIG. 5. Desaturation of procaryotic MGD by isolated chloroplasts as shown by separation of molecular species. Chloroplasts from pea (P.s.) and nightshade (S.n.) were incubated for 75 min in the MGD mode for subsequent isolation of acetate-labeled MGD. Molecular species of the purified MGD were separated by argentation TLC in chloroform: methanol:acetone:acetic acid (80:15:5:1, v/v). Radioautographically localized bands were scraped off for determination of % labeling. Identification of individual bands is based on sequence of species as analyzed previously (25), fatty acid analysis (see Figs. 2-4), and staining of unlabeled species with dichlorofluoresceine.

C18:3/C16:3 MGD. A convincing demonstration of similar reactions with 18:3 chloroplasts is still missing, if it does occur at all.

Acquisition of Eucaryotic MGD. So far we have shown that isolated chloroplasts, irrespective of their origin, synthesize de novo only procaryotic MGD. As outlined in the beginning, eucaryotic MGD can be synthesized by 16:3 as well as 18:3 chloroplasts only in cooperation with extraplastidic systems. To demonstrate this cooperative reaction, we isolated chloroplasts from leaves which had been incubated for 2.5 h with acetate. During this time, chloroplasts should have been provided with eucaryotic DAG for MGD biosynthesis. The in vivo-labeled chloroplasts were isolated and the extracted MGD subjected to the same analyses as described above.

MGD from in vivo-labeled pea chloroplasts showed a more complex pattern of molecular species (Fig. 6a) than MGD synthesized by isolated chloroplasts (Fig. 5). Most important is the presence of eucaryotic species with two C18-acyl chains at both positions which were missing in Figure 5. Fatty acid analysis showed the presence of 25% dienoic and 17% trienoic acids in contrast to the results of Figures 2 and 3. A positional analysis showed that the polysaturated acids were distributed nearly symmetrically between the C-1 and C-2 positions as observed with pea MGD (8). Similar experiments with Chenopodium and Solanum in which, for simplicity, MGD was directly isolated from acetate-labeled leaves gave the results shown in Figure 6b. Reversed-phase TLC of hydrogenated methyl esters obtained from C-1 and C-2 of MGD showed the presence of C18 fatty acids (32% in Chenopodium, 18% in Solanum) at the C-2 position which was occupied exclusively by C16 chains, when MGD was synthesized by isolated chloroplasts (Fig. 4). This supports the idea (18) that cooperation between chloroplasts and extraplastidic systems as occurring during in vivo labeling is necessary to produce C18/C18 MGD which is present in varying proportions in 16:3 as well as 18:3 chloroplasts.

Our results from experiments with isolated chloroplasts are summarized in Table II. Despite the more qualitative form of the statements, similarities as well as differences in the enzyme equipment of chloroplasts from different plants become evident. Future assays of individual enzymic reactions will be required to provide additional evidence on a quantitative basis. Our data indicate that
Fig. 6. Appearance of eucaryotic MGD in chloroplasts from 18:3 and 16:3 plants after acetate-labeling of leaves. Assignments of molecular species of MGD from pea chloroplasts (a) as separated by argentation TLC (cf. Fig. 5) were supported by positional analysis of constituent fatty acids. The presence of C18-fatty acids at C-2 of MGD from *Solanum nodiflorum* and *Chenopodium album* (b) was demonstrated by the sequence of lipase hydrolysis, hydrogenation, and reversed-phase TLC. The presence of C18-fatty acids at C-2 serves as criterion for the eucaryotic origin of DAG backbones (cf. Fig. 4). Abbreviations of plants as in Figure 2.

Table II. Reactions of Lipid Metabolism in Chloroplasts Isolated from 16:3 and 18:3 Plants

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Type of Chloroplasts</th>
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<tbody>
<tr>
<td>Fatty acid synthesis from acetate</td>
<td>16:3 ++ +</td>
</tr>
<tr>
<td>PA dephosphorylation</td>
<td>++ ++ +</td>
</tr>
<tr>
<td>Galactosylation of newly synthesized DAG</td>
<td>++ +</td>
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<tr>
<td>Galactosylation of preexisting DAG</td>
<td>+++++</td>
</tr>
<tr>
<td>De novo synthesis of eucaryotic MGD</td>
<td>ND* ND*</td>
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<tr>
<td>Desaturation of C18:1 at C-1 of MGD</td>
<td>+ + ND</td>
</tr>
<tr>
<td>Desaturation of C16:0 at C-2 of MGD</td>
<td>++ + ND</td>
</tr>
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</table>

*Not detected.*

chloroplasts from different groups of plants can be differentiated with regard to their synthetic capacities in lipid biosynthesis. This may be added to the well-known differences in the enzymic equipment of mesophyll chloroplasts from C3 and C4 plants. Chloroplasts from 16:3 plants possess the full equipment for synthesis and desaturation of procaroytic MGD, whereas this property is more or less lost in 18:3 plants. The residual capacity for synthesis of procaroytic PA may be required for the production of PG and SQD. On the other hand, both types of chloroplasts have the ability to assemble eucaryotic MGD. This explains the gradual change from pronounced 16:3 to pure 18:3 plants as evident from the decreasing proportion of C16:3 in MGD of different plants. From an evolutionary point of view, 18:3 chloroplasts may be considered as more integrated organelles which have abandoned more of their procaroytic features.

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**LITERATURE CITED**


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