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Free Fatty Acids Regulate Two Galactosyltransferases in Chloroplast Envelope Membranes Isolated from Spinach Leaves

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ABSTRACT

Effects of $MgCl_2$ and free fatty acids (FFA) on galactolipid:galactolipid galactosyltransferase (GGGT) and UDP-galactose:1,2-diacylglycerol galactosyltransferase (UDGT) in chloroplast envelope membranes isolated from spinach (*Spinacia oleracea* L.) leaves were examined. GGGT activity was sigmoidally stimulated by $MgCl_2$ with a saturated concentration of more than 5 millimolar. Free α-linolenic acid (18:3) caused a drastic increase in GGGT activity under limiting concentrations of $MgCl_2$, without affecting its maximum activity at higher $MgCl_2$ concentrations. Free 18:3 alone did not affect the GGGT activity. The effective species of FFA for the stimulation of GGGT activity in the presence of $MgCl_2$ were unsaturated 16- and 18-carbon fatty acids. GGGT activity was also stimulated by 18:3 in the presence of $MnCl_2$, $CaCl_2$ and a high concentration of KCl in place of $MgCl_2$. UDGT activity was hyperbolically enhanced by $MgCl_2$ with a saturated concentration of 1 to 2 millimolar. In contrast to GGGT, UDGT was severely inhibited by 18:3, and $MgCl_2$-induced stimulation was completely abolished by 18:3. Unsaturated 16- and 18-carbon fatty acids were more inhibitory to UDGT than the saturated acids. The dependence of GGGT activity on monogalactosyldiacylglycerol (MGDG) and $MgCl_2$ concentrations was identical in the envelope membranes isolated from non- and ozone (0.5 microliter/liter)-fumigated spinach leaves, indicating that GGGT remained active in the leaves during ozone fumigation. The results are discussed in relation to the regulation of galactolipid biosynthesis by the endogenous FFA in the envelopes and to the involvement of GGGT in the triacylglycerol synthesis from MGDG in ozone-fumigated leaves.

MGDG[1] and DGDG are the major polar lipid constituents of chloroplast membranes (thylakoids and envelopes), and their biosynthesis has been extensively studied (6, 14, 20). The final process of the galactolipid synthesis proceeds by two galactosylation steps of 1,2-DG to MGDG and MGDG to DGDG in the envelope membranes (6, 14). The former reaction is catalyzed by UDGT (5, 11, 19) and the latter by GGGT (4, 10, 26). GGGT can also synthesize TGDG and TTGDG, normally absent in plant leaves *in vivo* (5, 26).

In the preceding papers (22–24), we have shown that treatment of spinach plants with ozone, a major atmospheric pollutant, causes a drastic change in galactolipid composition in leaf tissues, suggesting that the enzymes responsible for the galactolipid metabolism are affected by ozone. We have also demonstrated that ozone stimulates the synthesis of TG from 1,2-DG moieties of MGDG, and that the conversion of MGDG to 1,2-DG is catalyzed primarily by GGGT (22–24). Therefore, it seems likely that ozone stimulates GGGT activity in spinach leaves. Evidence has been also presented that the release of FFA from complex lipids in chloroplasts is enhanced at early stages of ozone fumigation (22), indicating that the chloroplast envelopes would be exposed to increased levels of FFA during ozone fumigation.

Here we have tested the effect of FFA on galactolipid synthesizing enzymes, GGGT and UDGT, in the envelope membranes isolated from spinach chloroplasts. We found that FFA, in combination with divalent cations, stimulates GGGT activity, and that, in contrast, FFA has an inhibitory effect on UDGT activity. The mode of ozone action on the synthesis of TG from MGDG is discussed.

MATERIALS AND METHODS

Plant Materials and Ozone Fumigation

Spinach (*Spinacia oleracea* L., cv New Asia) plants were grown from seeds for 7 to 8 weeks under natural light in a glasshouse. The fumigation of the plants with ozone (0.5 μL/L) was carried out in an environment-controlled growth cabinet. Other growth and fumigation conditions were described previously (21).

Preparation of Envelope Membranes

Envelope membranes were prepared from intact chloroplasts based on the method of Douce and Joyard (8). The intact chloroplasts were isolated from spinach leaves (fresh weight of 300–400 g) by discontinuous Percoll gradients as described previously (22). The chloroplasts obtained, more than 90% intact (yield of 30–50 mg Chl), were ruptured by suspending (1–2 mg Chl/mL) in a medium containing 10 mM

[1] Abbreviations: MGDG, DGDG, TGDG, and TTGDG, mono-, di-, tri-, and tetragalactosyldiacylglycerol, respectively; 1,2-DG, 1,2-diacylglycerol; UDGT, UDP-galactose:1,2-diacylglycerol galactosyltransferase; GGGT, galactolipid:galactolipid galactosyltransferase; TG, triacylglycerol; FFA, free fatty acid; 16:3, hexadecatrienoic acid; 18:3, α-linolenic acid; 16:0, palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid.

Tricine (pH 7.6 with Tris) and 4 mM $MgCl_2$, and layered on top of the discontinuous sucrose gradients composed, from the bottom, of 0.93 M (10 mL) and 0.6 M (10 mL) sucrose in the same medium. After centrifugation at 72,000*g* for 1 h (RPS 27-2(3) rotor, Hitachi), the purified envelope membranes (yield of 1–3 mg protein) were recovered from the interface of the two sucrose layers. The envelope membranes were diluted with a medium of 25 mM Tricine (pH 7.2 with Tris) and 1 mM EDTA, and sedimented by centrifugation at 130,000*g* for 45 min. The membranes were finally suspended in a medium of 25 mM Tricine (pH 7.2 with Tris) and 0.1 mM EDTA, and either used for the experiments or immediately frozen at −80°C until they were used within a month. Chl content in the envelopes, a marker of contaminated thylakoids, was less than 0.5 μg/mg protein.

Preparation of MGDG and 16:3

MGDG was separated from the lipid extract of spinach leaves by preparative TLC on silica gel as described previously (24). For the preparation of free 16:3, the fatty acid methyl esters prepared by the methanolysis of spinach MGDG were dissolved in acetonitrile and separated by HPLC (Tri-Rotar, JASCO) with a reverse-phase column, Zorbax ODS (4.6 × 250 mm) and a mobile phase of acetonitrile at a flow rate of 0.8 mL/min. The separated methyl esters were detected with a UV spectrophotometer (UVIDEC-100-II, JASCO) at 192 nm. In some cases, the separated methyl-16:3 fraction was rechromatographed in the same system as above to remove contaminating traces of methyl-18:3. The methyl-16:3 obtained was more than 99% pure as judged by GLC. Free 16:3 was obtained from the methyl ester by saponification according to Bergelson (1).

Preparation of Labeled MGDG

[^{14}C-Gal]MGDG was prepared by the incubation of isolated envelopes (approximately 2 mg protein) in a medium containing 0.5 mM UDP-[^{14}C]Gal (4.9 μCi/μmol), 10 mM $MgCl_2$, and 20 mM Tricine-KOH (pH 7.2) at 30°C for 1 h. The lipids in the envelopes were extracted (2) and the labeled MGDG was purified as described previously (24). The MGDG obtained had a specific radioactivity of 1.86 μCi/μmol, which was diluted with spinach unlabeled MGDG to 0.22 μCi/μmol and used for the assay of GGGT activity.

Enzyme Assays

GGGT was assayed according to the method of Heemskerk *et al.* (10, 11) with slight modifications. Unless otherwise stated, the reaction mixture contained 25 mM Tricine (pH 7.2 with Tris), 0.2 mM [^{14}C]MGDG (10^4 dpm), 0.5 mM sodium deoxycholate, various concentrations of $MgCl_2$ and 18:3, and envelope membranes (20 μg protein) in a final volume of 100 μL. [^{14}C]MGDG was first dispersed in a solution of sodium deoxycholate with a sonicator (W-113, Honda Electric; 100 W). The molar ratio of galactolipid to detergent was 1:2.5. The radioactive solution was then mixed with envelope membranes and resonicated for 10 s at 4°C. The envelope membranes, thus loaded with [^{14}C]MGDG, were divided into test tubes, and then 18:3 in ethanol was added and quickly mixed with a vortex mixer at 4°C. Final concentration of ethanol in a reaction mixture was 1% (v/v), which had no effect on GGGT activity. The reaction was started 15 min after the addition of 18:3: the mixture was supplied with $MgCl_2$, followed by incubation at 30°C for 10 min with continuous shaking. The reaction was terminated by the addition of 1.8 mL chloroform/methanol (1:1, v/v), and the lipids were recovered by the procedure of Bligh and Dyer (2). The galactolipids were separated by silica gel TLC (0.25-mm thick, Merck) developed with acetone/benzene/water (91:30:8, v/v) and located with I_2 vapor and radiochromatogram scanner (LB 2723, Berthold). The silica gel zones containing galactolipids were scraped off the plate, and the radioactivity was determined by scintillation counting with Aquasol-2/methanol (2:1, v/v) according to Douce and Joyard (7). GGGT activity was determined by the amount of label converted to DGDG, TGDG, and TTGDG, and the amount of MGDG

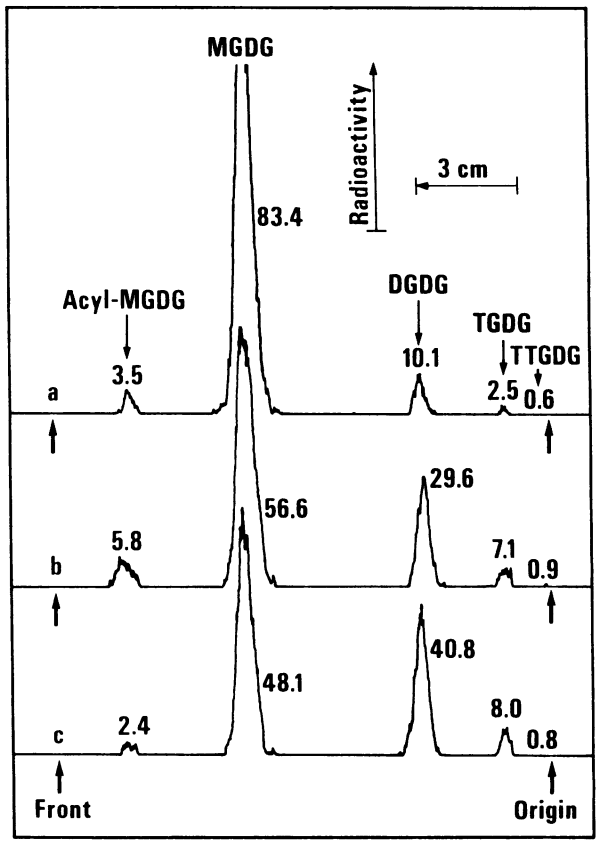

Figure 1. Metabolism of [^{14}C-Gal]MGDG in chloroplast envelope membranes as affected by $MgCl_2$ and 18:3. Envelope membranes (20 μg protein) were loaded with [^{14}C]MGDG (20 nmol, 2 × 10^4 dpm) in a medium of 25 mM Tricine (pH 7.2 with Tris) and 0.5 mM sodium deoxycholate. The envelope membranes were incubated with 2 mM $MgCl_2$ (a), 2 mM $MgCl_2$ and 300 μM 18:3 (b), and 10 mM $MgCl_2$ (c) at 30°C for 15 min. Traces represent radioscans of envelope lipids separated by silica gel TLC developed with acetone/benzene/water (91:30:8, v/v). After the scan, silica gel from labeled spots was scraped off and the radioactivity determined by scintillation counting. Numbers are the percent distribution of the label. Acyl-MGDG, 6-*O*-acyl-MGDG.

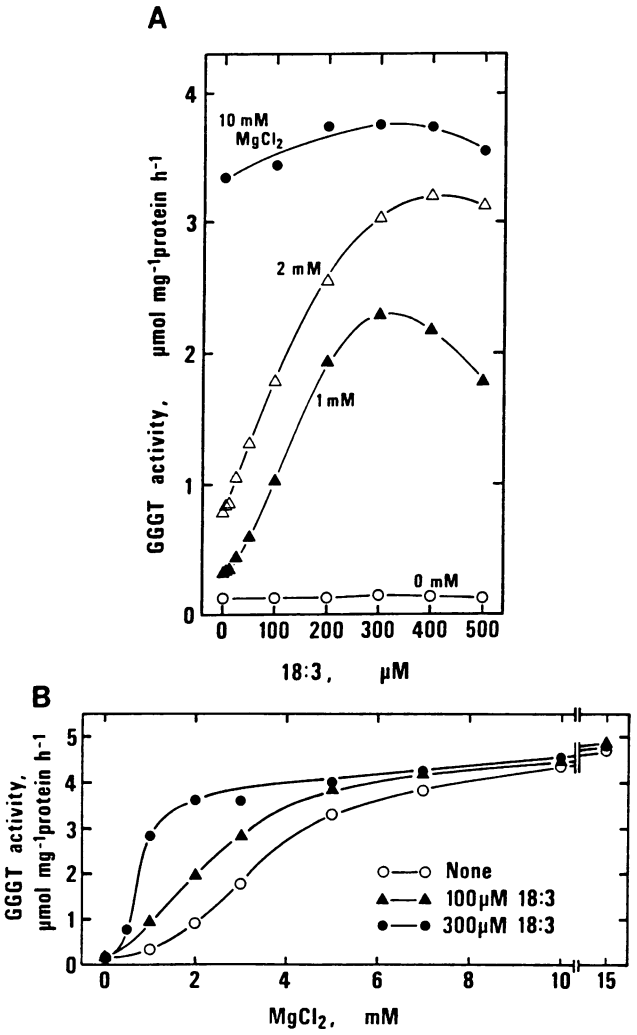

Figure 2. Dependence of GGGT activity on 18:3 and $MgCl_2$ concentrations in chloroplast envelope membranes. (A) GGGT was assayed with 0 (○), 1 (▲), 2 (△), and 10 (●) mM $MgCl_2$. (B) GGGT was assayed with 0 (○), 100 (▲), and 300 (●) µM 18:3.

metabolized was estimated after the correction was made for decreasing the specific radioactivity with endogenous MGDG in the envelope membranes (11, 12).

UDGT was assayed according to the method of Douce and Joyard (7) with some modifications. The reaction mixture was composed of 25 mM Tricine (pH 7.2 with Tris), 0.5 mM UDP-[^{3}H]Gal (16.4 µCi/µmol), various concentrations of $MgCl_2$ and 18:3, and envelope membranes (20 µg protein) in a total volume of 100 µL. Free 18:3 was added as described above. The reaction was initiated by the addition of, in succession, $MgCl_2$ and UDP-[^{3}H]Gal, followed by incubation at 30°C for 10 min with continuous shaking. The reaction was terminated by the addition of 1.8 mL chloroform/methanol (1:1, v/v), followed by the partitioning in chloroform and aqueous layers (2). The lower chloroform layer was washed twice with freshly prepared aqueous-layer solvent to remove traces of contaminated UDP-[^{3}H]Gal. The radioactive galactolipids were determined as described above.

Lipid and Protein Determination

Envelope lipids were extracted, separated by TLC and quantified by GLC (24). Envelope protein was measured according to Lowry *et al.* (16) with BSA as the standard.

Chemicals

UDP-[U-^{14}C]Gal (333 mCi/mmol), UDP-[4,5-^{3}H(N)]Gal (34.0 Ci/mmol) and Aquasol-2 were purchased from New England Nuclear. Percoll was obtained from Pharmacia Fine Chemicals. BSA (essentially fatty acid-free), UDP-Gal (Na salt) and FFA of 16:0, 16:1, 18:0, 18:1, 18:2, and 18:3 were obtained from Sigma.

RESULTS

Effects of $MgCl_2$ and 18:3 on GGGT Activity

Figures 1 and 2 show the metabolism of [^{14}C-Gal]MGDG in envelope membranes in the presence of $MgCl_2$ and 18:3. As reported by Heemskerk *et al.* (10, 11), GGGT-dependent syntheses of oligogalactolipids, especially DGDG and TGDG, from MGDG were enhanced at high concentrations of $MgCl_2$ (Figs. 1 and 2). When 18:3 was added together with $MgCl_2$, the galactose transfer by GGGT was markedly stimulated at low concentrations of $MgCl_2$ (Figs. 1 and 2). With 1 and 2 mM $MgCl_2$, the increase in the activity was obvious at less than 25 µM 18:3 and was maximal at 300 to 400 µM (Fig. 2A). Without $MgCl_2$, GGGT activity was low irrespective of the 18:3 concentration, and with 10 mM $MgCl_2$, the stimulation of GGGT activity was saturated and insensitive to 18:3 (Fig. 2, A and B).

The optimum pH for GGGT activity was around 7.0 in

Table I. *Effect of EDTA and BSA on the Stimulation of GGGT Activity by 18:3 and $MgCl_2$ in Envelope Membranes Isolated from Spinach Chloroplasts*

Expt.	Additives	GGGT
		µmol/mg protein/h
1	None	0.13
	18:3 (300 µM)	0.14
	EDTA (2 mM)	0.12
	EDTA (10 mM)	0.17
	BSA (2 mg/mL)	0.10
	BSA (20 mg/mL)	0.36
2	None	0.11
	$MgCl_2$ (2 mM)	0.90
	EDTA (2 mM), $MgCl_2$ (2 mM)	0.14
	EDTA (10 mM), $MgCl_2$ (2 mM)	0.16
	BSA (20 mg/mL), $MgCl_2$ (2 mM)	0.73
	18:3 (300 µM), $MgCl_2$ (2 mM)	3.12
	EDTA (2 mM), 18:3 (300 µM), $MgCl_2$ (2 mM)	0.55
	EDTA (10 mM), 18:3 (300 µM), $MgCl_2$ (2 mM)	1.20
	BSA (2 mg/mL), 18:3 (300 µM), $MgCl_2$ (2 mM)	1.16
	BSA (20 mg/mL), 18:3 (300 µM), $MgCl_2$ (2 mM)	0.73

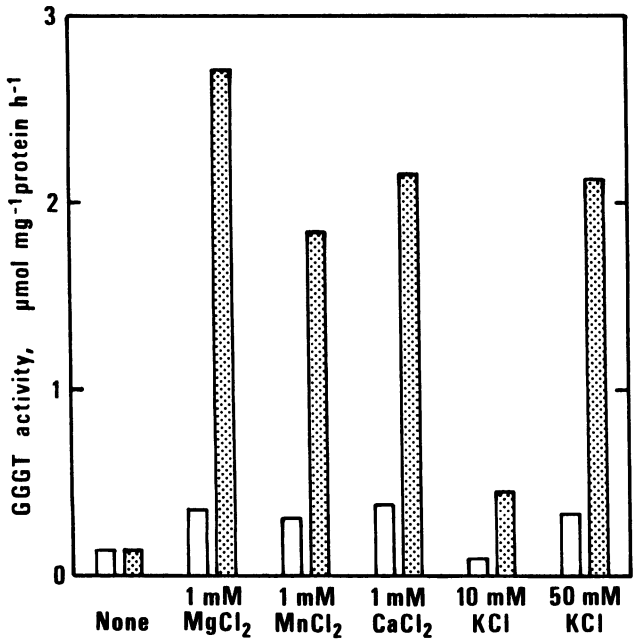

Figure 3. Influence of several kinds of salts on spinach envelope GGGT activity in the presence (*stippled bars*) and absence (*open bars*) of 300 μM 18:3. Reaction was started by the addition of the salts. Values are the average of two separate experiments.

the presence of 2 mM $MgCl_2$ with 300 μM 18:3 or of 10 mM $MgCl_2$ (data not shown). As the pH was lowered, the label was predominantly increased in 6-*O*-acyl-MGDG (data not shown), consistent with the result of Heemskerk *et al.* (10). In the assay at pH 7.2, however, the label incorporated into this lipid hardly exceeded 5% of the total label applied to the membranes (see Fig. 1).

EDTA reduced the $MgCl_2$-stimulated GGGT activity almost to background levels (Table I). However, in the presence of 18:3, EDTA suppressed the $MgCl_2$-stimulated GGGT activity to a lesser extent (Table I). BSA suppressed the 18:3, $MgCl_2$-stimulated GGGT activity at high levels (Table I), probably due to the removal of 18:3 from envelope membranes as a result of its binding to BSA.

Heemskerk *et al.* (10) found that GGGT activity is stimulated not only by $MgCl_2$ but also by several divalent cations and high concentrations of monovalent cations. Figure 3 shows that $MgCl_2$ can be replaced by $MnCl_2$, $CaCl_2$ and also by a high concentration of KCl for the stimulation of GGGT activity in combination with 18:3.

Fatty Acid Specificity for GGGT

The specificity of FFA for the stimulation of GGGT activity is shown in Figure 4. Unsaturated 16- and 18-carbon fatty acids were more effective than the corresponding saturated acids. We note here that the fatty acid specificity was quite similar to that for the acyl-CoA synthetase in spinach envelope membranes (15).

Effects of $MgCl_2$ and 18:3 on UDGT Activity

Without the addition of 18:3, incorporation of UDP-Gal into the galactolipids was almost fully stimulated at 2 mM $MgCl_2$ (Fig. 5); the activity was as high as 2 μmol/mg protein/h, which matched the activity as observed in spinach (5, 26) and pea (3) envelope membranes. The addition of 18:3 inhibited the incorporation of UDP-Gal over the range of the $MgCl_2$ concentrations tested and, in addition, abolished the stimulative effect of $MgCl_2$ on the activity (Fig. 5).

Since MGDG newly synthesized from UDP-Gal by the action of UDGT is then utilized for the substrate of GGGT within the membranes (26), the incorporation of UDP-Gal into galactolipids, as shown in Figure 5, is the result of cooperative action of both enzymes. Table II shows the distribution of label among the galactolipids incorporated from UDP-[^{3}H]Gal. Without $MgCl_2$, label was predominantly located in MGDG, indicating that GGGT was almost blocked. Nevertheless, the total incorporation was suppressed by 18:3, which is evidence that 18:3 inhibits UDGT. In the presence of low concentrations of $MgCl_2$ (1 and 2 mM), 18:3 not only suppressed the total galactose incorporation but also shifted the distribution of label to the oligogalactolipids by about 10% (see also Fig. 6). This is further evidence that 18:3 suppresses UDGT, but stimulates GGGT activities.

Fatty Acid Specificity for UDGT

The dependence of UDGT inhibition on 18:3 concentration is shown in the inset of Figure 6. The inhibition was

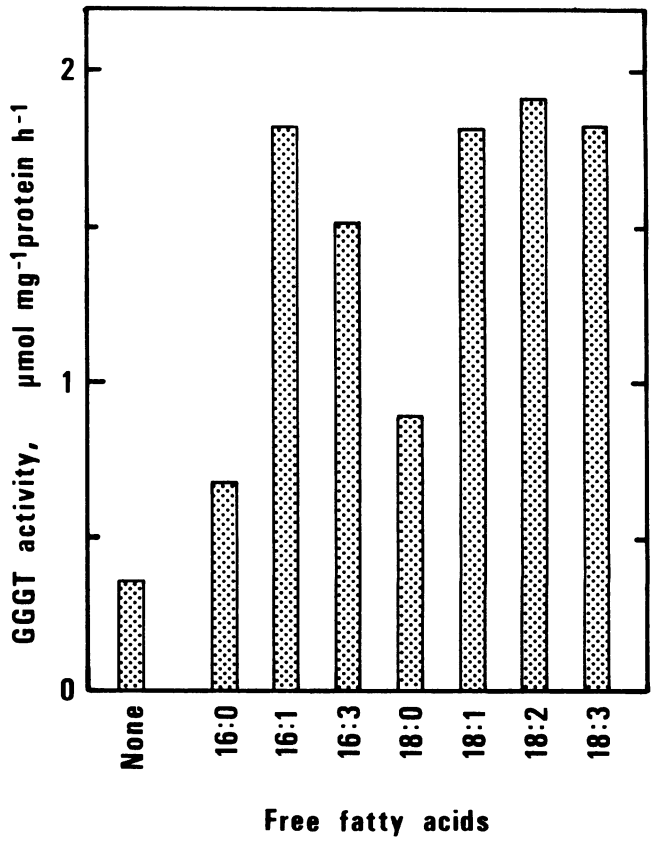

Figure 4. Effect of FFA species on GGGT activity in chloroplast envelope membranes. The activity was assayed with 300 μM of various kinds of FFA and 1 mM $MgCl_2$. Values are the average of two separate experiments.

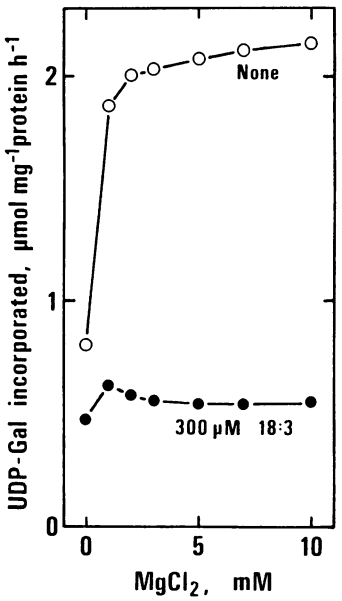

Figure 5. Effects of $MgCl_2$ and 18:3 on the incorporation of UDP-Gal into galactolipids in chloroplast envelope membranes. Envelope membranes (20 μg protein) were incubated at 30°C for 10 min with 0.5 mM UDP-[^{3}H]Gal and 300 μM 18:3 as indicated.

detectable with as little as 5 μM, giving a half-inhibition at about 100 μM. Figure 6 shows the fatty acid specificity of UDGT inhibition in the envelope membranes. Unsaturated 16- and 18-carbon fatty acid species were more inhibitory to UDGT than the corresponding saturated species. Again, the label was distributed more heavily in the oligogalactolipids than MGDG when UDGT was strongly inhibited by unsaturated fatty acids. A comparison of the fatty acid specificity for GGGT stimulation (Fig. 4) and UDGT inhibition (Fig. 6) shows that the same FFA species have opposite effects on two separate galactosyltransferases.

Effect of Ozone on GGGT

We have previously shown the operation of GGGT in ozone-fumigated spinach leaves based on the accumulation of TGDG and TTGDG in the leaves (22). Figure 7, A and B, shows the dependence of GGGT activity on the substrate (MGDG) and $MgCl_2$ concentrations, respectively, in the envelope membranes isolated from ozone-fumigated spinach leaves. The characteristics of GGGT were unaffected by ozone, confirming that GGGT remained active in the leaves during the fumigation with ozone.

DISCUSSION

In the previous paper (22), we reported that both the hydrolysis of MGDG to FFA, especially 18:3, and the formation of oligogalactolipids were stimulated in spinach leaves at early stages of ozone fumigation. The present results clearly demonstrated the stimulative action of FFA on GGGT activity in combination with divalent cations (Figs. 2 and 3). In addition, the effective species of FFA were shown to be unsaturated 16- and 18-carbon fatty acids (Fig. 4), including 18:3. Therefore, the oligogalactolipid formation induced by ozone can be ascribed to the enhancement of GGGT activity by FFA temporarily accumulated in the envelope membranes. However, we cannot rule out the other possibilities of ozone action, *e.g.* an increase in Mg^{2+} concentration around the enzyme, which also stimulates GGGT activity (Fig. 2).

In contrast to the lack of effect of ozone fumigation on the properties of GGGT in spinach chloroplast envelopes under the present conditions (Fig. 7), Mudd *et al.* (18) reported that incorporation of UDP-Gal into oligogalactolipids, GGGT activity, was inhibited by ozone bubbling into the suspension of spinach chloroplasts and suggested that hydrogen peroxide produced during the oxidation of fatty acids by ozone was responsible for GGGT inhibition. Thus, the lack of an effect of ozone on GGGT activity in our experiments (Fig. 7) would be accounted for by the absence of hydrogen peroxide production; little fatty acid oxidation was detected in spinach leaves at early stages of ozone fumigation (21, 23).

Fatty acid specificity for the stimulation of GGGT activity (Fig. 4) closely resembled the substrate specificity of acyl-CoA synthetase in the spinach chloroplast envelopes (15). This may be partly attributable to the difference in solubility among fatty acid species. Consequently, FFA temporarily accumulated in the envelopes causes an increased production of oligogalactolipids as well as 1,2-DG from MGDG by the enhancement of GGGT; FFA is concomitantly metabolized

Table II. *Effect of $MgCl_2$ and 18:3 on the Incorporation of Label from UDP-[^{3}H]Gal into Galactolipids in Envelope Membranes Isolated from Spinach Chloroplasts*

Values were obtained from the results given in Figure 5.

$MgCl_2$	18:3	Total Incorporation	Distribution of Label		
			MGDG	DGDG	TGDG + TTGDG
mM	μM	μmol/mg protein/h		%	
0	0	0.80	96.6	1.6	1.8
0	300	0.47	91.9	5.0	3.1
1	0	1.87	62.4	25.7	11.9
1	300	0.62	51.2	30.0	18.8
2	0	2.00	58.0	28.1	13.9
2	300	0.58	49.8	31.5	18.8
10	0	2.14	45.8	32.4	21.8
10	300	0.55	49.3	31.7	19.0

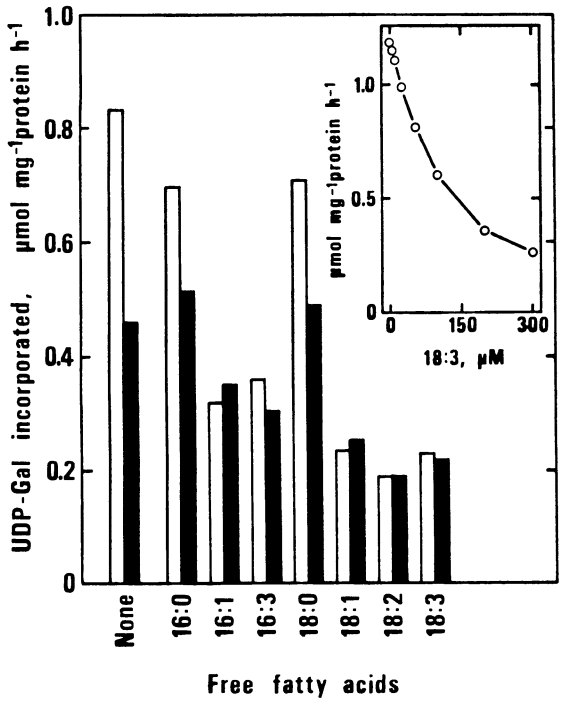

Figure 6. Effect of FFA species on UDGT activity in chloroplast envelope membranes. UDGT was assayed with envelope membranes (20 μg protein) in the presence of 2 mM $MgCl_2$ and 150 μM of various FFA species. Label in MGDG (*white bars*) and in the sum of DGDG, TGDG, and TTGDG (*black bars*) was determined as described in "Materials and Methods." *Inset* is the concentration dependence of UDGT inhibition by 18:3. $MgCl_2$, 2 mM.

to acyl-CoA by acyl-CoA synthetase in the envelopes. Consistent with this view, FFA, mainly 18:3, formed in the chloroplasts was shown to be preferentially converted to acyl-CoA by the addition of CoA and ATP (22). Thus, 1,2-DG and acyl-CoA produced in the envelopes would function as the substrates for TG synthesis. We have already presented evidence that 1,2-DG liberated from MGDG was further metabolized to TG by the acylation with 18:3 in ozone-fumigated leaves (22, 24).

In contrast to the stimulation of GGGT activity, FFA inhibited UDGT in the envelope membranes (Figs. 5 and 6; Table II). The inhibition of UDGT by 18:1 has been already reported (11, 27). This is favorable to the production of 1,2-DG from MGDG, but unfavorable to the reverse production of MGDG from 1,2-DG. It has been shown that incorporation of UDP-Gal into MGDG is gradually decreased, but incorporation into oligogalactolipids is steadily increased or accelerated when chloroplasts (9, 19) and envelopes (5) are incubated for prolonged periods. This observation may be partly accounted for by the inhibition of UDGT and the stimulation of GGGT activity by FFA, since FFA is known to be readily produced by aging of chloroplasts (13, 17, 22).

Although GGGT required higher Mg^{2+} concentrations than UDGT for its full activity, FFA lowered the Mg^{2+} concentration required for maximal GGGT activity, thus GGGT was active in the presence of FFA at similar ranges of Mg^{2+} concentrations to UDGT (Figs. 2B and 5). Since EDTA

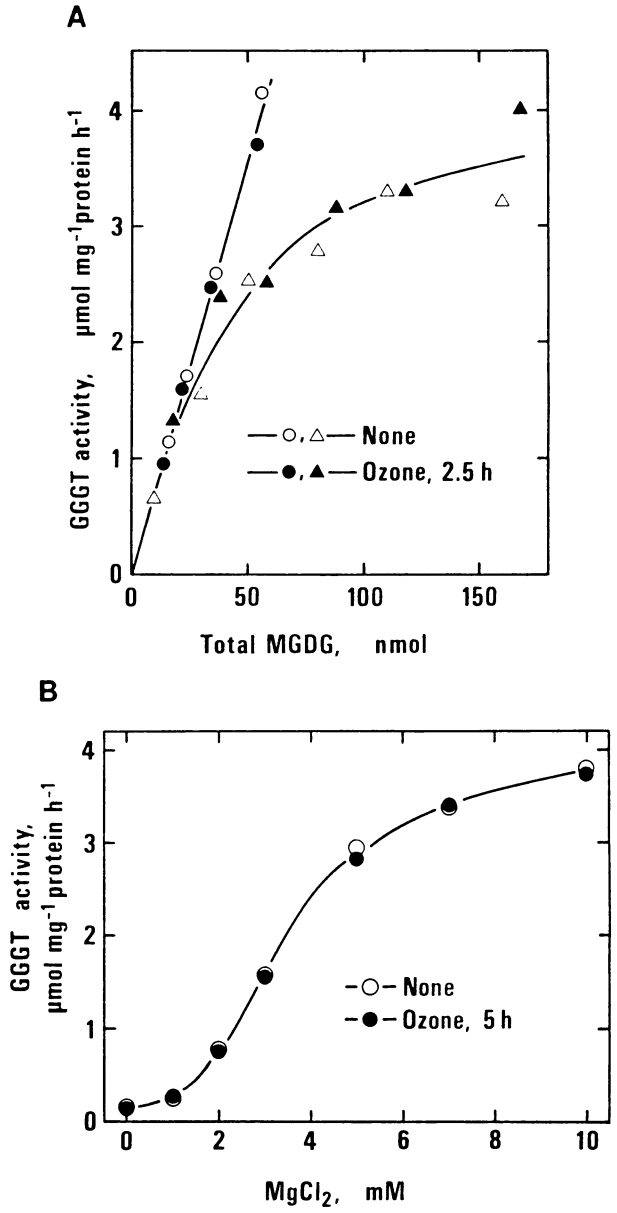

Figure 7. Effects of MGDG and $MgCl_2$ concentrations on GGGT activity in the envelope membranes isolated from ozone-fumigated spinach leaves. (A) The indicated amounts of labeled MGDG were first sonicated in aqueous sodium deoxycholate, followed by resonication with the envelope membranes. The final concentrations of sodium deoxycholate were 0.5 (○, ●) and 1.5 (△, ▲) mM, and the envelope membranes eq to 17.0 μg protein. The membranes were incubated with 10 mM $MgCl_2$ at 30°C for 15 min. (B) Envelope membranes (20 μg protein) were loaded with labeled MGDG (20 nmol, 10^4 dpm) and incubated with various concentrations of $MgCl_2$ at 30°C for 10 min. Sodium deoxycholate, 0.5 mM.

diminished the stimulation of GGGT (Table I; ref. 10) and UDGT (9) activities, free Mg^{2+} is expected to play a regulatory role in galactolipid biosynthesis in chloroplast membranes. The localization of GGGT in outer envelope membranes of spinach chloroplasts (4, 12) suggests that GGGT activity is regulated by cytosolic Mg^{2+}. Although UDGT is located in inner envelope membranes of spinach chloroplasts (4, 5), the fact that incorporation of UDP-Gal in intact spinach chloroplasts is markedly stimulated by exogenously applied $MgCl_2$ (9) may indicate that UDGT is also regulated by the cytosolic Mg^{2+}. Recently, free Mg^{2+} in cytosol of mung bean root tips was estimated to be 0.4 mM (28); this value is adequate for the regulation of both enzymes.

According to the current concept of lipid synthesis (20, 25), fatty acids *de novo* synthesized as acyl thioesters in the stroma are hydrolyzed to FFA when exported outside the chloroplasts. The FFA formed, mainly 18:1 and 16:0, pass through the inner envelope membranes and are converted to acyl-CoA in outer envelope membranes. Consequently, both inner and outer envelope membranes are continuously exposed to 18:1 and 16:0. As shown in Figures 4 and 6, 18:1 was an effective species for both the stimulation of GGGT and the inhibition of UDGT; 16:0 was less effective. Therefore, it is likely that both envelope enzymes are regulated by these fatty acids when they pass through the envelopes as intermediates of fatty acid synthesis.

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