Modulation of Fatty Acid Incorporation and Desaturation by Trifluoperazine in Fungi

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The effects of trifluoperazine (TFP) on [1-^{14}C]fatty acid incorporation into the lipids of *Mortierella ramanniana* var. *angulispora* were studied. TFP decreased [1-^{14}C]-fatty acid incorporation into phosphatidylcholine, phosphatidylethanolamine and triacylglycerol, but greatly increased ^{14}C-labeling in phosphatidic acid. These changes in [1-^{14}C]fatty acid incorporation induced by TFP were accompanied by a decrease in desaturation of some [1-^{14}C]fatty acids taken up by the fungal cells. When [1-^{14}C]lioleic acid (LA) was incubated with the fungal cells, total γ-linolenic acid (GLA) formation from incorporated [1-^{14}C]LA decreased, but the ^{14}C-labeled GLA content in individual lipid classes was essentially unchanged. This suggests that the site of the TFP effect on GLA formation from [1-^{14}C]LA taken up from the medium is not the desaturase acting on LA linked to complex lipids. On the other hand, GLA formation from [1-^{14}C]oleic acid was much less susceptible to TFP, which suggests that in this fungus Δ6 desaturation to GLA has at least two different pathways with different degrees of susceptibility to TFP.
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Although some fungi have long been known to contain polyunsaturated fatty acids (1,2), the mechanisms of their biosynthesis have remained unclear. Aerobic desaturation has been well characterized in oleic acid formation, where thioester derivatives are used as substrates. On the other hand, it has been shown that the direct desaturation of fatty acid bound to complex lipids occurs in eukaryotic microorganisms (3) and plants (4). However, only a few desaturases using acyl-CoA or acyl carrier protein (ACP) derivatives as substrates (5–7) and no desaturases using complex-lipid linked fatty acids have been purified to homogeneity, presumably because of the instability of the enzymes. Thus, the elucidation of the molecular nature of the desaturases awaits further investigation. A recent genetic approach may become a powerful tool to clarify the diversity of the desaturases (8–10).

Mortierella fungi have a high lipid content and contain polyunsaturated fatty acids such as γ-linolenic acid (GLA) (11,12), arachidonic acid (13,14) and eicosapentaenoic acid (15), depending on the species. In previous studies (16,17) we investigated regulatory mechanisms which may determine the GLA composition in individual lipid classes in *Mortierella ramanniana* var. *angulispora* and reported the differential synthesis of GLA between neutral lipids and phospholipids in the fungus. In the present study we tried to modulate lipid metabolism in this fungus to further clarify the relationship between fatty acid incorporation into lipid classes and its desaturation thereafter. For this purpose, we used trifluoperazine (TFP), which belongs to the class of the antipsychotic agents, phenothiazines, because TFP and its related phenothiazine drug, chlorpromazine, have been reported to modify glycerolipid metabolism in mammalian cells (18). In addition, Δ6 desaturation of exogenous [1-^{14}C]linoleic acid (LA) or [1-^{14}C]oleic acid (OA) was compared in regard to its susceptibility to TFP.

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Abbreviations: ACP, acyl carrier protein; DG, diacylglycerol; FFA, free fatty acid; GL, glycolipid; GLA, γ-linolenic acid; LA, linoleic acid; OA, oleic acid; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; TFP, trifluoperazine; TG, triacylglycerol; TLC, thin-layer chromatography.

MATERIALS AND METHODS

Materials. [1-^{14}C]Stearic acid (59 mCi/mmol), [1-^{14}C]OA (59 mCi/mmol) and [1-^{14}C]LA (59 mCi/mmol) were obtained from New England Nuclear (Boston, MA). Unlabeled stearic acid, OA, LA, GLA, TFP, chlorpromazine, cerulenin, ouabain, cytochalasin B and colchicine were purchased from Sigma (St. Louis, MO). Silica gel G thin-layer chromatographic (TLC) plates were obtained from Merck (Darmstadt, Federal Republic of Germany), and KC18 (reversed phase) TLC plates were acquired from Whatman (Maidstone, U.K.). All solvents were of reagent grade.

Microorganisms and culture conditions. Mortierella ramanniana var. *angulispora* (IFO 8187) was obtained from the culture collection of the Institute of Fermentation (Osaka, Japan). The fungi were maintained on a yeast-extract/malt-extract agar medium. The liquid medium contained glucose, inorganic salts and vitamins, as described previously (16).

Incorporation of ^{14}C-labeled fatty acids into fungal lipids and extraction of lipids. ^{14}C-Labeled fatty acids were incorporated as described previously (17), except that several drugs were added. One mL of fungal cell culture grown in rotary shakers (180 rpm) at 30°C for one day, when cells were at the exponential growth phase, were incubated with 3.4 μM (0.2 μCi/mL) [1-^{14}C]fatty acids at 30°C for 0.5–4 hr in the presence or absence of TFP or other drugs. Since these drugs were dissolved in ethanol, control experiments were performed at a final ethanol concentration of 1% (v/v). After incubation, the fungal cells were cooled on ice and washed with 1 mL of 0.1 M phosphate buffer (pH 6.0), followed by centrifugation (1000 g, 5 min) to remove ^{14}C-labeled fatty acids not taken up by the fungal cells. Lipids were extracted from the fungal cell pellets with 3 mL of chloroform/methanol (1:2, v/v). After 1 hr, 1 mL of chloroform and 1 mL of 0.1 M phosphate buffer were added. The upper aqueous layer was washed twice with 1 mL of chloroform, and the lower chloroform layers wre collected.

Lipid analysis. Lipids were analyzed as described previously (17). For fatty acid analysis, extracted lipids were transmethylated and the resultant fatty acid methylesters were separated by reversed phase TLC on KC18 plates. Neutral lipid classes and polar lipid classes were separated by TLC on Silica gel 60 plates. When

necessary, two-dimensional TLC was performed for checking ^{14}C-labeled TLC fractions of polar lipids. ^{14}C-Labeled spots were detected by autoradiography and scraped into scintillation vials. Radioactivity was determined with a Beckman liquid scintillation system (model LS1701; Beckman, Fullerton, CA) with automatic quenching correction.

Other methods. The dry cell weight and total lipid content were measured by weight as described previously (16). Lactate dehydrogenase was assayed as described by Kornberg (18).

RESULTS

Effects of TFP on [1-^{14}C]LA incorporation and desaturation. TFP changed the [1-^{14}C]LA incorporation into individual lipids in this fungus as shown in Table 1. Though TFP inhibited cell proliferation of the fungus, the fungal cells kept their cell integrity as judged by the retention of the cytosolic marker enzyme, lactate dehydrogenase, up to 3×10^{-4} M TFP. When the fungal cells, having been treated with TFP, were washed to remove TFP and were incubated in normal medium, they resumed proliferation. Modification of the [1-^{14}C]LA incorporation profile by TFP was similar to that described in rat hepatocytes (19). ^{14}C Incorporation into triacylglycerol (TG), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) was decreased by TFP, whereas ^{14}C incorporation into phosphatidic acid (PA) was increased. These results were quite evident from the dose dependence of the TFP effects on the ^{14}C incorporation into individual lipids (Fig. 1). Figure 1 also shows the order in which ^{14}C incorporation into individual lipids was decreased in response to an increase in TFP concentration. ^{14}C Incorporation into PC or PE was most susceptible to TFP; ^{14}C incorporation into TG, phosphatidylserine (PS), PA and diacylglycerol (DG) was decreased by TFP in this order. TFP also caused accumulation of [^{14}C]FFA (free fatty acids which meant that exogenous [1-^{14}C]LA was not utilized for incorporation into individual complex lipids. Since TFP did not affect *in vitro* acyl-CoA synthetase activity (data not shown), the increase in [^{14}C]-FFA may not be caused by direct inhibition of utilization of fatty acids, but by inhibition of the transport of exogenous fatty acids in the fungal cells. Table 2 shows the time course of ^{14}C incorporation into individual lipids with or without 10^{-4} M TFP. TFP affected ^{14}C incorporation into PC, PS and PA at the early stages of incubation irrespective of whether it increased or decreased

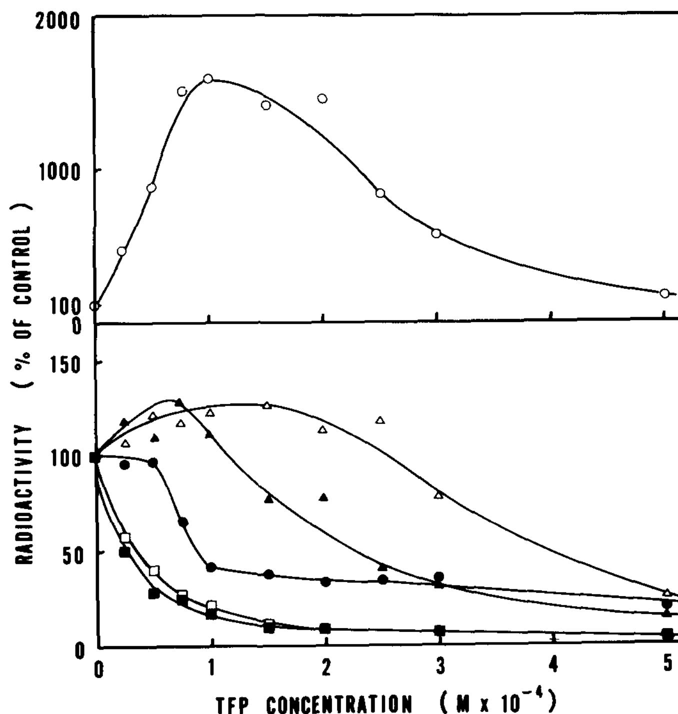

FIG. 1. Dose dependence of TFP effects on [1-^{14}C]LA incorporation into major lipid classes. [1-^{14}C]LA was incubated with fungal cells for 1 hr. [1-^{14}C]LA incorporation into TG (●), DG (△), PC (■), PE (□), PS (▲), and PA (○) was changed by raising the TFP concentration. Values are expressed as percentages of radioactivities incorporated in the absence of TFP (means of duplicates).

TABLE 1

Effects of TFP on [1-^{14}C]LA Incorporation into Various Lipid Classes[a]

Lipids[b]	^{14}C Incorporation (DPM × 10^{-3}/mg DCW[c]) Control	10^{-4}M TFP	5×10^{-4}M TFP
TG	54.2	26.3	5.6
DG	3.3	4.2	0.6
FFA	65.0	84.2	203.9
PC	46.7	17.3	2.8
PE	21.8	11.0	1.7
PS	10.9	15.2	3.8
PA	0.9	10.7	2.3
GL	4.2	1.3	0.2
PI	3.0	2.0	0.5
Total	221.0	201.0	234.5

[a]Values are means of triplicates. [1-^{14}C]LA were incubated with fungal cells for 1 hr.

[b]There were some unidentified spots in which ^{14}C incorporation was increased by TFP. TFP also modified [1-^{14}C]LA incorporation into sterol esters, which caused a slight change of the Rf value of sterol esters.

[c]DCW, dry cell weight.

TABLE 2

Effects of TFP on Time Course of [1-^{14}C]LA Incorporation[a]

	^{14}C Incorporation (DPM × 10^{-3}/mg DCW[b])					
	Control			10^{-4} M TFP		
Lipids	0.5 hr	1 hr	4 hr	0.5 hr	1 hr	4 hr
TG	22.3	55.3	118.9	19.5	22.2	30.3
DG	2.1	2.6	3.1	1.3	2.1	4.4
FFA	117.4	115.7	88.3	121.4	155.5	123.7
PC	23.4	36.2	34.4	14.8	16.3	13.1
PE	10.2	15.7	18.1	88.8	10.3	9.0
PS	6.9	6.7	4.5	13.2	10.4	6.4
PA	0.7	0.7	0.8	9.5	8.6	8.0
GL	1.9	2.5	4.5	1.1	1.2	4.5
PI	1.3	2.3	1.9	1.5	1.6	1.5
Total	118.6	241.3	278.9	193.5	231.2	200.9

[a]Values are means of duplicates.

[b]DCW, dry cell weight

incorporation. On the other hand, ^{14}C incorporation into TG, DG and PE was greatly affected at the later incubation times. Since ^{14}C incorporation into PA initially increased so rapidly and then decreased after longer incubations, we suggest that the initial ^{14}C-labeled PA was not derived from the degradation of ^{14}C-labeled TG, PC and PE, but accumulated because of inhibition of *de novo* lipid synthesis via PA.

When Δ6 desaturation of [1-^{14}C]LA to [^{14}C]GLA in the total lipids of the fungus was observed by a similar experiment, TFP lowered GLA formation from LA, as is shown in Table 3. The dose dependent decrease in GLA formation from LA caused by TFP is also shown in Figure 2. Chlorpromazine, a phenothiazine drug, caused similar inhibitory effect on Δ6 desaturation, although cerulenin, an inhibitor of fatty acid synthetase, did not affect GLA formation. Drugs which were assumed to interact with the plasma membrane or cytoskeleton did not affect GLA formation.

TABLE 3

Effects of Various Drugs on GLA Formation from [1-^{14}C]LA[a]

Drugs		GLA formation[b] (%)
Control		14.5 ± 1.5 (100%)
Trifluoperazine	10^{-4} M	4.8 ± 0.2 (33%)
	5 × 10^{-4} M	1.4 ± 0.3 (10%)
Chlorpromazine	10^{-4} M	11.5 ± 0.9 (79%)
	5 × 10^{-4} M	2.6 ± 0.4 (17%)
Cerulenin	10^{-4} M	13.4 ± 0.1 (92%)
	10^{-3} M	13.8 ± 1.3 (95%)
Ouabain	10^{-4} M	14.0 ± 0.5 (96%)
	10^{-3} M	13.7 ± 0.1 (95%)
Cytochalasin B	10^{-4} M	16.3 ± 0.8 (112%)
	10^{-3} M	14.0 ± 0.3 (97%)
Colchicine	10^{-4} M	14.3 ± 0.6 (99%)
	10^{-3} M	13.8 ± 1.1 (95%)

[a][1-^{14}C]LA was incubated with fungal cells for 1 hr.

[b]Values represent the [^{14}C]GLA content in total lipids after each drug treatment (means of triplicates ± S.D.).

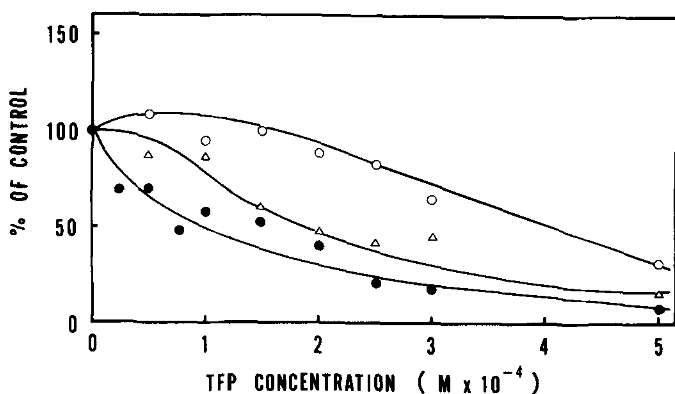

FIG. 2. Dose dependence of TFP effects on the desaturation from [1-^{14}C]LA or [1-^{14}C]OA. After [1-^{14}C]LA or [1-^{14}C]OA was incubated with fungal cells for 1 hr, the extracted lipids were transmethylated and radioactivities of the resultant methyl esters of OA, LA and GLA were determined. Effects of TFP on the conversion from [1-^{14}C]LA to [^{14}C]GLA (●), from [1-^{14}C]OA to [^{14}C]GLA (○), and from [1-^{14}C]OA to [^{14}C]LA (△) were evaluated as percentages of that in the absence of TFP. Values are means of duplicates.

To examine how the decrease in GLA formation in total lipids influenced the distribution of ^{14}C-labeled GLA in individual lipids, we measured the relative amount of ^{14}C-labeled GLA in the total radioactivity incorporated into each lipid (Table 4). Though slight changes of ^{14}C-labeled GLA content in individual lipids were observed with changes in TFP concentration, these could not account for the total decrease in GLA formation caused by TFP. The results suggest that the site of the TFP effect on Δ6 desaturation was not desaturation which acted on LA incorporated into individual lipids, but desaturation which acted on LA prior to being incorporated into complex lipids.

Effects of TFP on [1-^{14}C]stearic acid or [1-^{14}C]OA incorporation. To examine whether the decrease in LA desaturation due to TFP was also observed with other fatty acids, the effects of TFP on [1-^{14}C]stearic acid and [1-^{14}C]OA incorporation were also analyzed (Table 5). Table 5 shows that the desaturation of [1-^{14}C]stearic acid taken up into fungal cells was decresed by 10^{-4} M TFP, whereas the desaturation of [1-^{14}C]OA taken up was not affected by 10^{-4} M TFP. In particular, GLA formation from [1-^{14}C]OA added to the medium was unaffected by 10^{-4} M TFP, which was different from GLA formation from [1-^{14}C]LA added to the medium. Therefore, Δ6 desaturation and GLA formation from [1-^{14}C]OA may have a pathway different from that of added [1-^{14}C]LA.

Figure 2 also shows the dose dependency of the effects of TFP on the desaturation from [1-^{14}C]OA. Compared with the Δ6 desaturation from exogenous [1-^{14}C]LA, Δ6 desaturation from exogenous [1-^{14}C]OA was much less susceptible to TFP. At a concentration of 2 × 10^{-4} M TFP, Δ6 desaturation from added [1-^{14}C]OA was not decreased. TFP dependence of Δ12 desaturation of added [1-^{14}C]OA was quite similar to that of Δ6 desaturation of added [1-^{14}C]LA.

The effects of TFP on [1-^{14}C]OA incorporation into various lipids are shown in Table 6. The effects of TFP were similar to those on [1-^{14}C]LA incorporation (Table 2). ^{14}C Incorporation into TG, PC and PE was

TABLE 4

[^{14}C]GLA Content of Major Lipid Classes After TFP Treatment for One Hour

	[^{14}C]GLA content[a] (%)			
		TFP		
Lipids	Control	10^{-4}M	2 × 10^{-4}M	5 × 10^{-4}M
TG	10.9 ± 1.5 (100%)	11.9 ± 0.5 (109%)	11.2 ± 1.4 (103%)	12.6 ± 1.5 (116%)
PC	30.2 ± 1.2 (100%)	33.6 ± 1.4 (111%)	32.1 ± 7.6 (106%)	27.3 ± 3.2 (90%)
PE	45.4 ± 1.6 (100%)	42.1 ± 1.0 (93%)	39.0 ± 0.5 (86%)	41.7 ± 3.5 (92%)
PS	40.9 ± 2.2 (100%)	38.6 ± 2.3 (94%)	32.4 ± 7.9 (79%)	37.5 ± 6.2 (92%)
PA	—[b]	38.7 ± 2.7	33.2 ± 5.1	30.1 ± 2.3

[a]Values represent the [^{14}C]GLA content in each lipid class (means of triplicates ± S.D.). Values in parentheses represent percent of control in each lipid class.

[b]Not tested because ^{14}C-labeled PA was trace.

TABLE 5

Effects of TFP on the Desaturation of [1-^{14}C]Stearic Acid or [1-^{14}C]Oleic Acid[a]

Incorporated fatty acid	Addition	Time (hr)	[^{14}C]Fatty acid (%) 18:0	18:1	18:2	18:3[b]	Total ^{14}C (DPM × 10^{-4})
[1-^{14}C]Stearic acid	none	1	89.4	7.4	2.0	1.2	19.8
		4	84.3	10.7	3.1	1.9	20.2
	10^{-4}M TFP	1	91.9	4.8	2.0	1.3	15.6
		4	92.1	4.8	1.9	1.2	18.3
[1-^{14}C]Oleic acid	none	1	1.0	86.2	5.7	7.1	19.5
		4	1.5	79.5	7.5	11.5	18.8
	10^{-4}M TFP	1	0.9	84.8	5.2	9.1	15.0
		4	1.3	80.0	6.6	12.0	15.9

[a]Values are means of duplicates. [b]GLA.

TABLE 6

Effects of TFP on [1-^{14}C]OA Incorporation Into Various Lipid Classes[a]

Lipids	^{14}C Incorporation (DPM × 10^{-3}/mg DCW[b]) Control	10^{-4}M TFP	5 × 10^{-4}M TFP
TG	37.9	24.0	2.0
DG	3.1	4.1	0.3
FFA	119.3	123.2	157.3
PC	11.5	12.3	0.2
PE	10.6	3.0	0.3
PS	4.6	4.7	0.6
PA	0.4	6.8	1.4
GL	1.1	0.6	0.3
PI	1.1	0.6	0.1
Total	196.7	189.0	175.2

[a]Values are means of duplicates. [1-^{14}C]OA was incubated with fungal cells for 1 hr.
[b]DCW, dry cell weight.

decreased, whereas ^{14}C incorporation into PA, PS and DG was increased.

DISCUSSION

The present study shows that in fungi the amphiphilic, cationic drug TFP modulates fatty acid incorporation into individual lipid classes as well as fatty acid desaturation. TFP decreased ^{14}C-labeled fatty acid incorporation into PC, PE and TG, but increased ^{14}C incorporation into PA. This was similar to the effect of phenothiazine drugs observed in mammalian cells (19–21). Another phenothiazine drug, chlorpromazine, showed similar tendencies, as did TFP (data not shown). One explanation for the modulation of fatty acid incorporation by amphiphilic phenothiazine drugs has been the inhibition of phosphatidate phosphohydrolase (20). In rat hepatocytes, chlorpromazine has been suggested to block the association of this enzyme with the membrane, thus preventing its transition to an active form (20). Other explanations which focussed on the inhibition of PC synthesis were proposed for HeLa cells (22) and GH_3 pituitary cells (23). In HeLa cells, CTP:phosphocholine cytidylyltransferase was shown to be inhibited by TFP and chlorpromazine, which would account for the decrease in PC synthesis. In GH_3 pituitary cells, TFP was shown to stimulate degradation of PC and sphingomyelin, which may cause an apparent decrease of [^{3}H]choline incorporation into these lipids.

From the dose dependence of the TFP effect on lipid metabolism in *Mortierella* fungi, differences in [^{14}C]fatty acid incorporation into individual lipid classes were distinct at a concentration of less than 2–3 × 10^{-4} M TFP. At higher levels, TFP gradually casued non-specific cell damage accompanied by a decrease in [^{14}C]fatty acid incorporation into all lipid classes (Fig. 1). Thus, we focused on the TFP effect at concentrations of less than 2–3 × 10^{-4} M. In these concentration ranges, [^{14}C]fatty acid incorporation into PC and PE was more susceptible to TFP than incorporation into TG (Fig. 1). Moreover, a slight increase in [^{14}C]fatty acids incorporation into DG by TFP was observed. These results suggest that inhibition of phosphatidate phosphohydrolase mainly occurs in this fungus because of the large accumulation of ^{14}C-label in PA, while factors other than reduced DG availability for PC, PE and TG synthesis may be involved in the decreased synthesis of these lipids. Increased degradation of PC, PE and TG due to TFP appears less likely, because increased ^{14}C incorporation into DG due to TFP was apparent at longer incubation times, whereas the decrease of ^{14}C-incorporation, especially into PC and PE, occurred early in the experiments (Table 2).

Another aspect of the phenothiazine effect reported for mammalian cells was accumulation of labeled acidic phospholipids such as phosphatidylinositol (PI) and phosphatidylglycerol (PG) upon labeled fatty acid incorporation (19,24). In the fungus, a slight accumulation of ^{14}C-label in PS was observed at the expense of PI and PG. Though little is known about the lipid metabolism in *Mortierella* fungi, it may be similar to that known for the lower eukaryote, *S. cerevisiae,* which has been extensively studied. In *S. cerevisiae,* the synthesis of phospholipids may be similar to that in higher eukaryotes, except for the synthesis of PS (25). PS is synthesized from CDP-DG and serine in *S. cerevisiae* (26), whereas in mammalian cells (27) PS is synthesized by a base exchange reaction involving PE. Thus, the differences in PS biosynthetic pathways between the mammalian cells and the

eukaryotic microorganisms probably contributed to the difference in ^{14}C accumulation in acidic phospholipids induced by amphiphilic cationic drugs.

Recently, TFP was reported to modify acyltransfer to phospholipids and cholesterol in fibroblasts (28). In this case, TFP enhanced [^{14}C]fatty acids incorporation into total phospholipids, which was not observed in the fungus. However, [^{14}C]fatty acid incorporation into sterol esters was modified by TFP in the fungus.

There have been several reports on inhibitors of desaturases. Substituted pyridazinones are well known to inhibit desaturase activities in higher plants (29,30) and in animals (31). The agents have been reported to directly affect the enzymes and to selectively act on certain desaturases, *e.g.*, the Δ15 desaturase in higher plants (32). Though TFP is known to interfere with lipid metabolism as mentioned above, a lowering of the desaturase activity by TFP has not previously been reported.

TFP caused no significant changes in the ^{14}C-labeled GLA content of individual lipid classes when the fungal cells were incubated with [1-^{14}C]LA (Table 4). This suggests that the site of action of TFP on the total Δ6 desaturation activity from exogenous [1-^{14}C]LA is not the Δ6 desaturase. An increase or decrease in the ^{14}C-labeled GLA content would occur in certain lipid classes if this type of Δ6 desaturase exists in the fungus, as it does in higher plants (33), and is inhibited by TFP. Δ6 Desaturation of exogenous LA, with a CoA derivative as substrate, may occur in the fungus. To confirm this possibility, one would need to follow changes in acyl-CoA labelling in further experiments. On the other hand, we have shown that ^{14}C-labeled GLA produced from [1-^{14}C]-LA exists in esterified rather than free form (16). This suggests that if Δ6 desaturation uses a CoA derivative as substrate, specific acyl transfer may be followed by Δ6 desaturation to incorporate GLA into specific lipids as obsessed in Δ12 desaturation (34). Thus, TFP may act on fatty acid transfer and Δ6 desaturation.

GLA formation from [1-^{14}C]OA was not changed at higher TFP concentrations, which suggests that sequential desaturation uses a different pool. Varying responses of different pathways of desaturation at the same chain position have been suggested from the differential effects of the compound, BASF 13-338 in *Arabidopsis* (30), in which α-linolenic acid in monogalactosyldiacylglycerol and in PC were produced under different controls. This raises the possibility that different pathways may exist for Δ6 desaturation in this fungus, *i.e.*, one which uses an LA derivative not linked to complex lipids as a substrate is responsible for the Δ6 desaturation from LA taken up from the medium, and the other which uses LA linked to complex lipids as a substrate is responsible for the Δ6 desaturation from LA derived from OA within the fungal cells. Though there is evidence which supports the existence of desaturases acting on complex lipid-linked fatty acids in microorganisms (35,36), animals (37) and plants (33,38–42), it is still unknown whether these desaturase systems work in *Mortierella* fungi.

It has been proposed that in higher plants OA in PC is desaturated to LA or GLA, which are channeled into the acyl-CoA pool by the reverse reaction of an acyl-CoA:lysophosphatidylcholine acyltransferase. The acyl-CoA thus generated is utilized in TG synthesis (43). In borage seeds, this mechanism may regulate the GLA composition of TG (44). In the fungus, we have shown that LA, which has been esterified into phospholipids such as PC, PE and PS, is readily desaturated to GLA, which is then transferred to TG (17). These results appear consistent with the proposed mechanism in higher plants, although there is no *in vitro* evidence as yet which suggests that this mechanism is also operative in this fungus. Thus, TFP might exert its effect on Δ6 desaturation by blocking some steps in the above scheme. If TFP modulates fatty acid specificity of lysophospholipid acyltransferase(s) so that exogenous [1-^{14}C]OA is preferentially utilized for desaturation as compared to exogenous [1-^{14}C]LA, the difference in TFP susceptibility between exogenous [1-^{14}C]OA desaturation and exogenous [1-^{14}C]LA desaturation will become interpretable. In addition to the possibilities described above, TFP may directly act on the desaturase enzyme(s).

TFP is widely known to block the action of Ca^{2+}-calmodulin (45). However, it remains to be seen whether the TFP effect on lipid metabolism is connected to the action of Ca^{2+}-calmodulin. It is presently not known whether acyltransferases or fatty acid desaturases are affected by Ca^{2+}-calmodulin.

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