Plant Physiol. (1988) 88, 56-60
0032-0889/88/88/0056/05/$01.00/0

Acyl Coenzyme A Preference of the Glycerol Phosphate Pathway in the Microsomes from the Maturing Seeds of Palm, Maize, and Rapeseed[1]

Received for publication January 20, 1988 and in revised form April 4, 1988

CHAO SUN, YI-ZHI CAO, AND ANTHONY H. C. HUANG*[2]
Biology Department, University of South Carolina, Columbia, South Carolina 29208

ABSTRACT

The acyl coenzyme A (CoA) preference of the glycerol phosphate pathway in the microsomes from the maturing seeds of palm (*Butia capitata* Becc.), maize (*Zea mays* L.), and rapeseed (*Brassica napus* L.) was tested. Each microsomal preparation was incubated with [^{14}C-U]-glycerol-3-phosphate and either lauroyl CoA, oleoyl CoA, or erucoyl CoA, and the ^{14}C-lipid products were separated and quantitated. In the presence of oleoyl CoA, the microsomes from each of the three species produced lysophosphatidic acid, phosphatidic acid, diacylglycerol, and triacylglycerol with kinetics consistent with the operation of the glycerol phosphate pathway. In the presence of erucoyl CoA, the microsomes from all the three species did not produce di- or tri-acyl lipids. In the presence of lauroyl CoA, only the microsomes from palm, but not those from maize or rapeseed, synthesized di- and tri-acyl lipids. This lack of reactivity of lauroyl CoA was also observed in the microsomes from maturing castor bean, peanut, and soybean. In maize seed and rapeseed, but not palm seed, the kinetics of labeling suggest that lauroyl and erucoyl moieties of the acyl CoAs were incorporated into lysophosphatidic acid but failed to enter into phosphatidic acid and thus the subsequent lipid products. We propose that the high degree of acyl specificity of lysophosphatidyl acyltransferase is the blocking step in the synthesis of triacylglycerols using lauroyl CoA or erucoyl CoA. The significance of the findings in seed oil biotechnology is discussed.

In oil seeds, the FA[3] composition of the storage TG is species- and variety-specific, and it can be modified by environmental factors such as temperature (14, 18). Within a seed species, the FA composition in each of the three positions of a TG is also largely inherited (18).

In seeds as well as in mammalian tissues, TG is synthesized from acyl CoA and glycerol-P via at least four enzyme reactions in the glycerol-P pathway (Kennedy pathway). The first two enzymes are glycerol-P acyltransferase and LPA acyltransferase. These two enzymes possess some acyl CoA preference, especially the second enzyme, such that their specificities as well as the *in vivo* pool sizes of acyl CoAs produce the observed positional acyl specificity in the TG (1, 14, 18, 19). The third enzyme is PA phosphatase, and its substrate specificity as far as the acyl moiety is concerned is unknown (18). The last enzyme, DG acyltransferase, is supposed to be relatively less specific for acyl CoAs, and it is assumed that the *in vivo* pool sizes of the acyl CoAs largely determine the acyl moiety in the *sn*-3 position of the TG.

In seed oil biotechnology, one major goal is to alter the chain length of the fatty acyl moiety of the TG (7, 13, 18). Researchers are using different approaches to manipulate the genes controlling enzymes for the elongation of FA. This manipulation appears to be theoretically workable. However, what is not known is whether the newly designed FA can be accommodated by the other components of the TG synthesis machinery. The acyl preference of all the three glycerol acyltransferases in maturing seeds of different species had been studied only with physiological substrates of palmitoyl CoA and C-18 acyl CoAs having 0-3 double bonds (4, 8, 11, 12, 18). Their activities on shorter or longer nonphysiological acyl CoAs had not been known.

Recently, we reported that DG acyltransferase from *Cuphea* and maize can utilize either lauroyl or oleoyl CoA, but not erucoyl CoA (5). The enzyme from rapeseed can utilize all the above three acyl CoAs. The implication of these findings is that the enzyme from different seed species can utilize acyl CoAs of physiological or shorter nonphysiological FA, but not of longer nonphysiological FA. We have extended our studies of acyl preference to the whole glycerol-P pathway, which involves four or more enzymes. Again, we used three selected oil seeds, palm, maize, and rapeseed, which have unique and very contrasting acyl moieties in the TG. We used palm endosperm instead of *Cuphea* cotyledon as the tissue that contains high amounts of lauric acid in TG, because we could obtain maturing palm seeds easily and in large quantities.

MATERIALS AND METHODS

Plant Materials. The oil storage tissues of maturing seeds at a stage when the fresh weights were approximately half of the values in the mature seeds were used. The scutella of inbred maize (*Zea mays* L. cv Mo-17), the cotyledons of rapeseeds (*Brassica napus* L. var. Dwarf Essex and var. Tower), the cotyledon of soybean (*Glycine max* L. merr cv Coker 237), and the endosperm of castor bean (*Ricinus communis* L. var. Hale) were obtained from plants grown in the greenhouse. Palm (*Butia capitata* Becc.) seeds were collected from a local plant, and the endosperm was used. The cotyledons of peanut (*Arachis hypogaea* L.) of unknown variety were obtained from plants grown at a local farm.

Preparation of Microsomes. All operations were performed at 0 to 4°C. The tissues were chopped with a razor blade in grinding medium in a Petri dish and then homogenized gently with a mortar and pestle. The grinding medium contained 1 mM EDTA, 10 mM KCl, 1 mM $MgCl_2$, 2 mM DTT, 0.6 M sucrose, and 0.15

[1]Supported by National Science Foundation grant DMB 85-15556.

[2]Present address: Department of Botany and Plant Sciences, University of California, Riverside, CA 92521.

[3]Abbreviations: FA, fatty acid(s); LPA, lysophosphatidic acid; PA phosphatidic acid; PC, phosphatidylcholine; DG, diacylglycerol(s); TG triacylglycerol(s).

M Tricine-HCl buffer (pH 7.5). The homogenate was filtered through a Nitex cloth (20 × 20 μm). The filtrate was centrifuged at 10,000*g* for 15 min, and the supernatant was recentrifuged at 100,000*g* for 100 min. The pellet was resuspended in a small volume of grinding medium. This microsomal preparation was either used immediately or frozen at −70°C until use.

Enzyme assays. The enzymic conversion of radioactive glycerol-3-P to lipids was assayed at 32°C for durations specified in "Results." Unless otherwise specified, the reaction mixture contained, in a final volume of 0.5 mL, 0.15 M 3-(*N*-morpholino)propanesulfonic acid-KOH (pH 7.2), 5 mM $MgCl_2$, 0.1% BSA, 50 μL microsomal preparation, 0.5 mM glycerol-3-P-[^{14}C-U](1 μCi, from New England Nuclear, Inc.), and 0.1 mM acyl CoA (lauroyl CoA, oleoyl CoA, and erucoyl CoA, from Sigma Corp.). This reaction mixture followed that described earlier (11) with modifications. The reaction was initiated by adding the microsomal preparation to the mixture. The reaction was stopped by adding 0.5 mL 0.4 M HCl. Four milliliters of chloroform/methanol (2:1, v/v) were then added, and the mixture was shaken vigorously. The mixture was centrifuged for 5 min at three-quarters full speed with an IEC HN-SII centrifuge. The lower phase was collected and mixed with lipid standards (50 μg each of dioleoyl PA, dipalmitoyl PA, oleoyl LPA, 1,2-diolein, and triolein). It was washed with 2.5 ml 0.1 M HCl/methanol (1:1, v/v), and the lower phase was collected after centrifugation and evaporated to dryness under a stream of nitrogen. The residues were redissolved with 250 μL chloroform and were divided into two equal halves for the separation of neutral lipids and phospholipids by TLC. The chloroform-lipids were applied to TLC plates (Whatman K-6 silica gel plates) and chromatographed in hexane/diethyl ether/ acetic acid (50/50/1, v/v/v) for the separation of neutral lipids, and in chloroform/methanol/ water/acetic acid (85/15/3.5/1, v/v/v/v) for the separation of phospholipids. The silica spots corresponding to the various lipids were visualized with iodine or by radioautography and were scraped into scintillation vials and counted for ^{14}-C.

RESULTS

Experimental Design. Seeds of three species were selected because of their content of unique and diverse FA in the storage TG. Maize kernel TG contain about 50% linoleic acid and 30% oleic acid (9). Rapeseed (*B. napus* L. var Dwarf Essex) has about 40% erucic acid, 15% linoleic acid, and 15% oleic acid in the seed TG (9, 16). Several palm species are known to contain a high percentage of lauric acids in the seed TG (9). We chose a palm (*B. capitata* Becc.) from a local city street to do our studies. In our gas chromatographic analysis, seed lipid bodies isolated from this palm contained about 41% lauric acid, 18% oleic acid, 12% myristic acid, 10% palmitic acid, 10% linoleic acid, and 7% capric acid.

The microsomal fractions from the endosperm of palm, the cotyledons of rapeseed, and the scutella of maize of maturing seeds were used as the sources of enzymes. It is known that the microsomes from maturing seeds of many species contain the entire glycerol-P pathway for the synthesis of TG from glycerol-3-P (18). In our studies, we applied [^{14}C-U]glycerol-3-P and either lauroyl CoA, oleoyl CoA, or erucoyl CoA to the microsomes, and we observed the formation of products of the glycerol-P pathway.

We used the microsomes from two varieties of rapeseed, the normal rapeseed containing a high percentage of erucic acid (variety Dwarf Essex) and an erucic acid-free rapeseed (variety Tower). The microsomes from both varieties exhibited very similar patterns of activities. These similarities include the kinetics of labeling in the presence of oleoyl CoA and the failure of incorporating erucoyl moiety of erucoyl CoA into TG (data not shown). Because of the availability of the microsomes from variety Tower during the experimental period, we used this variety to do the direct comparative studies in connection with the palm and maize microsomes, and we present the data on variety Tower only in this report.

Kinetics of Incorporation of [^{14}C]Glycerol-3-P into ^{14}C-lipids Using Oleoyl CoA as the Acyl Group Donor. Using oleoyl CoA as the acyl group donor, glycerol-3-P is converted to LPA, PA, DG, PC, and TG according to the known glycerol-P pathway (Fig. 1). The kinetics of labeling were similar among the three microsomal preparations. Little LPA accumulated. In maize and rapeseed, PA was produced in high amounts initially, and it then decreased concomitant with the increases in DG and TG. In palm, PA continued to accumulate while the production of DG and TG followed; no PC was produced.

The observed kinetics of labeling of the various lipids are in accord with the operation of the glycerol-P pathway reported earlier (17, 18). In addition, they are also in agreement with the activities of the individual enzymes detected *in vitro* (12). Little LPA accumulated in the reaction mixtures (Fig. 1). This is consistent with the findings that *in vitro*, LPA acyltransferase activity is about 10 times higher than glycerol-P acyltransferase activity (12). Substantial amounts of PA and DG accumulated before TG (Fig. 1). Again, this is consistent with the reports that *in vitro*, DG acyltransferase activity is two orders of magnitude less than LPA acyltransferase activity (12).

Oleic acid is an abundant FA in the TG of all the three seed species. In addition, in maize and rapeseed, a sizable percentage of oleic acid is present in each of the *sn*-1, -2, and -3 positions of the total TG (3). Thus, it is no surprise that oleoyl moiety of oleoyl CoA was incorporated into all three sn positions in TG by the microsomes from the three seed species.

The microsomal activities in the conversion of oleoyl CoA and glycerol-3-P to total lipids were as follows: 39 nmol/h·g fresh weight in palm, 100 nmol/h·g fresh weight in maize, and 354 nmol/h·g fresh weight in rapeseed. The maize microsomal activity is used as an example to compare this *in vitro* activity with the *in vivo* rate of lipid synthesis. The observed activity in the maize microsomes (2.1 mg/day·g fresh weight) in the synthesis of total lipids is less than, but within the same order of magnitude as, the *in vivo* rate of lipid synthesis (10–12 mg triolein/d·g fresh weight, 4). This *in vitro* activity being lower than the *in vivo* activity is likely due to the following factors. (a) We used 0.5 mM instead of a higher concentration of glycerol-3-P in the assay in order to increase the specific radioactivity. This concentration was not sufficient to saturate the reaction. In separate experiments using 0.1, 0.3, 0.5, and 0.7 mM of glycerol-3-P, we found that 0.7 mM was still not sufficient to saturate the reaction carried out by the microsomes from each of the three species (data not shown). (b) We used 0.1 mM instead of a higher, saturating concentration of oleoyl CoA in the assay. This concentration is not sufficient to saturate the various acyltransferase reactions (4, 5, 12, 17, 18). Because of the presence of 5 mM Mg^{2+} in the reaction mixture, oleoyl CoA at concentrations of 0.2 mM or higher in the present assay mixture would precipitate out (5, 6, 15). (c) Our gentle homogenization of the tissues and the subcellular fractionation procedure should not have recovered all the endoplasmic reticulum (microsomes). (d) It is likely that a suitable combination of different acyl CoAs to satisfy the different acyl CoA preference of the acyltransferases would generate a higher activity. For example, maize DG acyltransferase is twice as active on linoleoyl CoA as on oleoyl CoA (4).

Acyl CoA Specificity of the Glycerol-P Pathway in Palm Microsomes. The acyl CoA preference of the glycerol-P pathway in palm microsomes was studied using increasing concentrations of lauroyl CoA, oleoyl CoA, or erucoyl CoA in the presence of [^{14}C]glycerol-3-P (Fig. 2). A reaction time of 60 min was chosen. At 60 min, the reaction was still in progress (Fig. 1), and the

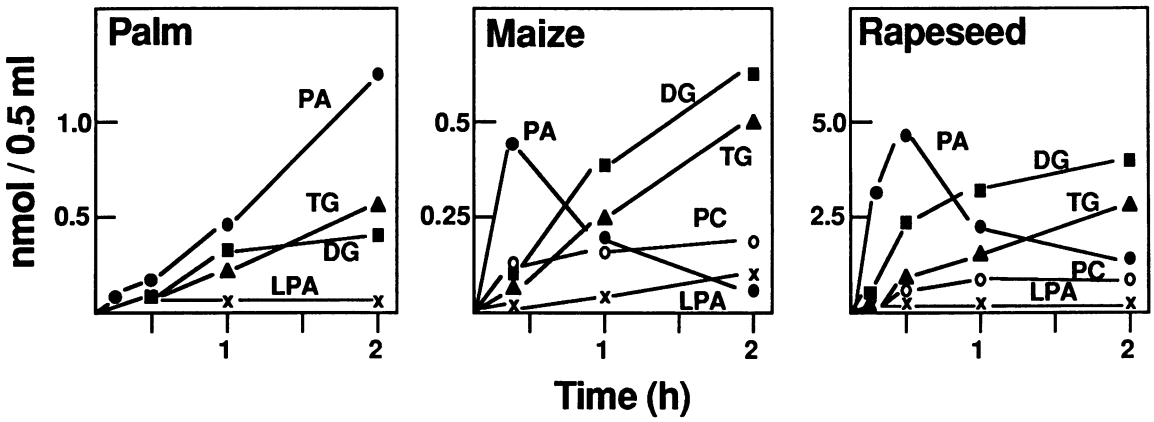

FIG. 1. Kinetics of labeling of the various glycerolipids from [^{14}C-]glycerol-3-P (0.5 mM) and oleoyl CoA (0.1 mM) by microsomes from the maturing seeds of various species. Microsomes from palm (0.31 mg protein), maize (0.20 mg) and rapeseed (0.29 mg) were used in a reaction mixture of 0.5 mL.

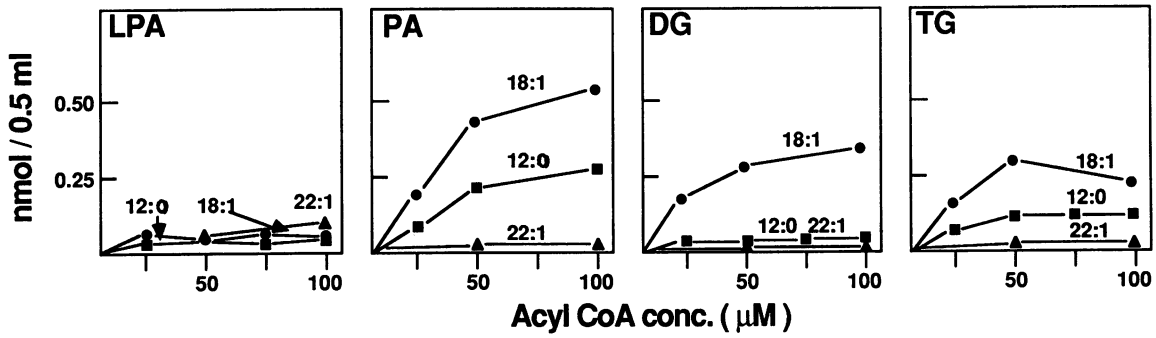

FIG. 2. Labeling of the various glycerolipids from [^{14}C]-glycerol-3-P (0.5 mM) and increasing concentrations of different acyl CoAs by palm microsomes. Microsomes containing 0.31 mg protein were used in a reaction mixture of 0.5 ml, and the reaction was allowed to proceed for 1 h; 12:0, lauroyl; 18:1, oleoyl; 22:1, erucoyl.

production of TG had not leveled off. Each of the three acyl CoAs generated a measurable but low amount of LPA. Either lauroyl CoA or oleoyl CoA but not erucoyl CoA, generated PA, DG, and TG. No PC was produced. Oleoyl CoA was roughly twice as active as lauroyl CoA in generating PA, DG, and TG.

It is known that in oil palm, each of the three *sn* positions in the total TG possesses a substantial percentage of lauroyl moiety (2). Therefore, successful incorporation of the lauroyl moiety into the TG in our studies using palm microsomes was not unexpected.

Acyl CoA Specificity of the Glycerol-P Pathway in Maize Microsomes. The acyl CoA specificity of the glycerol-P pathway in maize microsomes was studied similarly (Fig. 3). Each of lauroyl CoA, oleoyl CoA, and erucoyl CoA generated a low but measurable amount of LPA. However, only oleoyl CoA generated the subsequent reaction products, including PA, DG, PC, and TG.

Acyl CoA Specificity of the Glycerol-P Pathway in Rapeseed Microsomes. The acyl CoA specificty of glycerol-P pathway in rapeseed microsomes was studied similarly (Fig. 4). Again, each of lauroyl CoA, oleoyl CoA, and erucoyl CoA generated a low but measurable amount of LPA. However, only oleoyl CoA, and not even erucoyl CoA, generated the subsequent reaction products, including PA, DG, and TG.

Erucoyl CoA was not reactive with rapeseed microsomes in generating TG, even though about 40% of the acyl moiety in rapeseed TG is erucic acid. This finding should not be surprising, since the *sn*-2 position of rapeseed TG conspicuously lacks erucic acid (16). As judged from the synthesis of LPA but not PA, erucoyl CoA apparently was incorporated into the *sn*-1 position of glycerol-3-P, but failed to enter the *sn*-2 position, and thus the *sn*-3 position also.

In our studies of the normal rapeseed (variety Essex and the erucic acid-free rapeseed (variety Tower), the acyl specificity of the various enzymes behaved very similarly. The similarities include (a) the kinetics of formation of PA, DG, PC, and TG from oleoyl CoA and glycerol-3-P in the microsomal preparations, (b) the failure of the microsomal preparations to incorporate erucoyl moiety of erucoyl CoA into glycerol-3-P to produce di- and tri-acyl lipids, (c) the ability of DG acyltransferase to incorporate erucoyl moiety of the acyl CoA into the *sn*-3 position of TG (5), and (d) the diverse acyl specificity of the seedling lipases, including the ability to hydrolyze trierucin (10). These similarities reaffirm the fact that erucic acid-free rapeseed was obtained through breeding by eliminating the enzymes for the elongation of fatty acid from C-18 to C-22; apparently, the acyl specificity of the enzymes related to seed TG metabolism is not altered.

The observation that rapeseed and maize microsomes did not produce PA and other PA-derived lipids from erucoyl CoA or lauroyl CoA is taken as evidence that the activity of acyl CoA:PC acyltransferase in the reaction mixture was not detectable. Otherwise, the enzyme would have generated reactive acyl CoA such as oleoyl CoA from the microsomal lipids, such that radioactive PA and other PA-derived lipids would have been produced. Since this was not so, the radioactive LPA generated should represent the LPA containing the authentic acyl group of the added acyl CoA.

Lack of Reactivity of Lauroyl CoA in Producing DG and TG from Glycerol-3-P by Microsomes from Various Seed Species.

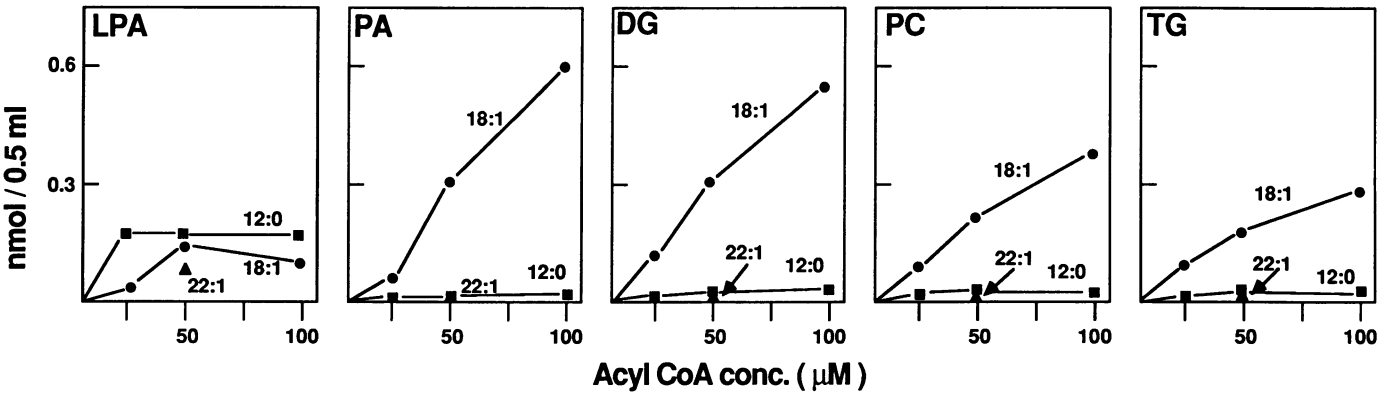

FIG. 3. Labeling of the various glycerolipids from [^{14}C]glycerol-3-P (0.5 mM) and increasing concentrations of different acyl CoAs by maize microsomes. Microsomes containing 0.20 mg protein were used in a reaction mixture of 0.5 mL, and the reaction was allowed to proceed for 1 h; 12:0, lauroyl; 18:1, oleoyl; 22:1, erucoyl.

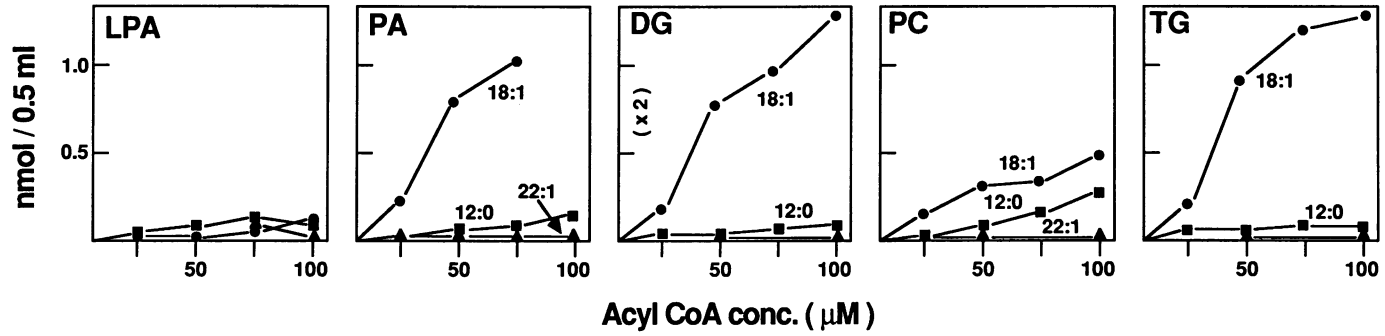

FIG. 4. Labeling of the various glycerolipids from [^{14}C]glycerol-3-P (0.5 mM) and increasing concentrations of different acyl CoAs by rapeseed microsomes. Microsomes containing 0.29 mg protein were used in a reaction mixture of 0.5 mL, and the reaction was allowed to proceed for 1 h; 12:0, lauroyl; 18:1, oleoyl; 22:1, erucoyl.

Table I. *Conversion of [^{14}C]Glycerol-3-P in the Presence of Either Lauroyl CoA or Oleoyl CoA to [^{14}C]PA, PC, DG and TG by Microsomes from Maturing Seeds of Various Species*

The results are expressed as ratios of the sum of the above ^{14}C lipid products generated in the presence of lauroyl CoA (0.1 mM) to those in oleoyl CoA (0.1 mM).

Species and Tissues	Lauroyl CoA/ Oleoyl CoA
	%
Maize scutellum	4
Palm endosperm	48
Rapeseed cotyledon	2
Soybean cotyledon	3
Peanut cotyledon	6
Castor bean endosperm	8

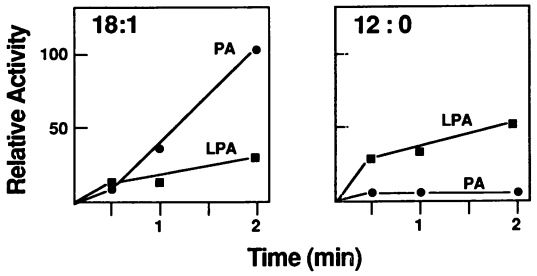

FIG. 5. Kinetics of short-time labeling of the various glycerolipids from [^{14}C]glycerol-3-P (0.5 mM) and various acyl CoAs (0.1 mM) by maize microsomes. The glycerolipids produced from oleoyl CoA (18:1) and lauroyl CoA (12:1) are shown; no glycerolipids were produced from eruoycl CoA.

DG acyltransferase in microsomes from *Cuphea* as well as maize and rapeseed can transfer the lauroyl moiety of lauroyl CoA into the *sn*-3 position of DG to form TG (5). In the current studies of microsomes from maize and rapeseed, lauroyl CoA apparently could incorporate into the *sn*-1 position of glycerol-3-P, but failed to enter into the *sn*-2 position, and thus the *sn*-3 position also. Similarly, the microsomes from soybean cotyledon, peanut cotyledon, and castor bean endosperm of maturing seeds could incorporate oleoyl moiety of oleoyl CoA into PA, DG, and TG, but failed to do so with lauroyl CoA (Table I). Apparently, only the microsomes from palm endosperm were able to transfer the lauroyl moiety of lauroyl CoA into the *sn*-2 position of acylglycerols.

Production of LPA from Glycerol-3-P and Lauroyl CoA, Oleoyl CoA, or Erucoyl CoA. As judged from the kinetics of labeling (Fig. 1) and the acyl CoA specificity (Fig. 2–4), the microsomal preparation from palm, maize, or rapeseed produced roughly equal amounts of LPA from glycerol-3-P with lauroyl CoA, oleoyl CoA, or erucoyl CoA. These amounts represent the steady state levels of LPA as an intermediate of the glycerol-P pathway. Among the three microsomal preparations, maize microsomes produced more LPA relative to the other lipid products. We used maize microsomes to study the kinetics of incorporation of acyl moieties from lauroyl CoA, oleoyl CoA, and erucoyl CoA into radioactive glycerol-3-P to form LPA and PA at short time durations. To increase the sensitivity of the assays, we used glycerol-3-P of higher specific radioactivity and lower concentration (0.62 mM containing 0.9 μCi in 0.5 mL reaction

mixture), and we scanned the radioautographs for the amounts of radioactivities after a long (3 weeks) exposure of the TLC plates. Figure 5 shows the data. In the presence of oleoyl CoA, LPA was initially labeled and it gradually leveled off; PA started to accumulate after an initial lag phase. In the presence of lauroyl CoA, LPA was labeled and it gradually leveled off to an amount higher than the corresponding oleoyl-LPA; no PA was produced. In the presence of erucoyl CoA, no LPA or PA was produced during the assay period (2 min). The findings show that the relative rate of incorporation of acyl moieties from the three acyl CoAs into glycerol-3-P is oleoyl CoA (LPA + PA) > lauroyl CoA (LPA) > erucoyl CoA. In addition, they reaffirm the idea that the lauroyl moiety of lauroyl CoA enters the *sn*-1 position of glycerol-3-P, but fails to incorporate into the *sn*-2 position.

DISCUSSION

A major objective of the current study was to determine if the glycerol-P pathway in the microsomes from various seed species can accept acyl CoA of nonphysiological shorter or longer fatty chain length. The information is important in the biotechnological modification of fatty acid composition of seed oils. It is clear that the microsomal enzymes from diverse seed species cannot utilize the nonphysiological long erucoyl CoA to synthesize TG. This lack of activity occurs even in the microsomes from *Brassica* seeds which contain a high percentage of erucic acid in the seed TG. In *Brassica* seeds, the acyl specificity of LPA acyltransferase is such that erucic acid is selectively excluded from the sn-2 position of the TG, whereas glycerol-P acyltransferase and DG acyltransferase can transfer erucic acid into the *sn*-1 and *sn*-3 positions, respectively. Continuous breeding of rapeseed for higher erucic acid content has approached the theoretical limit of 66% (16).

The microsomes from various seeds, except palm seed, cannot utilize the nonphysiological short lauroyl CoA to produce TG. This was a slight surprise. DG acyltransferase from various seed species can utilize acyl CoAs of physiological or the nonphysiological shorter lauric acid (5). In addition, the lipases from various seed species generally can hydrolyze TG containing the physiological or nonphysiological shorter fatty moieties (10). As judged from the entry of lauroyl moiety from lauroyl CoA to LPA but not PA, the blocking occurs at the LPA acyltransferase step. This enzyme is known to be the most acyl-specific acyltransferase among the three glycerol acyltransferases (1, 18, 19), and our current findings show that it even does not act on lauroyl CoA.

A major need in the current seed oil biotechnology is to modify the fatty acid moieties of TG from C-16 and C-18 to C-12 and C-10 in common agricultural oilseed crops. The present findings offer several strategical considerations. First, the fatty acid synthetase system should not be modified to a point such that only short (lauric) fatty acids are produced. In order for the seed to synthesize TG successfully, both short and long fatty acid should be present. Second, since the *sn*-2 position in TG cannot be occupied by lauric acid, theoretically only two-thirds of the total fatty acids in the seed TG can be short (lauric) fatty acid. If TG containing more than two-thirds of short (lauric) fatty acid is to be synthesized, or if the fatty acid synthetase can only be modified totally to synthesize short (lauric) fatty acid, genetic engineering of LPA acyltransferase from palm or *Cuphea* into the targeted seed crop is required. This latter manipulation will increase substantially the effort in the genetic engineering, since it will involve the successful genetic engineering of two presumably unrelated genes.

Acknowledgment—We thank Dr. K. C. Oo for information on the fatty acid and triacylglycerol composition of palm kernel oil, and V. Hollowell and C. Aulbach-Smith for collecting palm seeds locally.

LITERATURE CITED

1. BELL RB, RA COLEMAN 1983 Enzymes of triacylglycerol formation in mammals. *In* PD Boyer, ed, The Enzymes, Vol 16, Lipid Enzymology. Academic Press, New York, pp 87–111
2. BEZARD JA 1971 The component triglycerides in palm kernel oil. Lipids 6: 630–634
3. BROCKERHOFF H, M YURKOWSKI 1966 Stereospecific analyses of several vegetable fats. J Lipid Res 7: 62–64
4. CAO YZ, AHC HUANG 1986 Diacylglycerol acyltransferase in maturing oil seeds of maize and other species. Plant Physiol 82: 813–820
5. CAO YZ, AHC HUANG 1987 Acyl Coenzyme A preference of diacylglycerol acyltransferase from the maturing seeds of *Cuphea*, maize, rapeseed, and canola. Plant Physiol 84: 762–765
6. CONSTANTINIDES PP, JM STEIM 1986 Solubility of palmitoyl-Coenzyme A in acyltransferase assay buffers containing magnesium ions. Arch Biochem Biophys 250: 267–270
7. GRAHAM SA, F HIRSINGER, G RÖBBELEN 1981 Fatty acids of *Cuphea* (Lythraceae) seed lipids and their systematic significance. Am J Bot 68: 908–917
8. GRIFFITHS G, AK STOBART, S STYMNE 1985 The acylation of sn-glycerol-3-phosphate and the metabolism of phosphatidate in microsomal preparations from the developing cotyledons of safflower (*Carthamus tinctorius* L.) seed. Biochem J 230: 379–388
9. HILDITCH TP, PN WILLIAMS 1964 The Chemical Constitutions of Natural Fats. John Wiley & Sons, New York
10. HUANG AHC 1987 Lipases. In PK Stumpf, EE Conn, eds, The Biochemistry of Plants, Vol 9, Lipids. Academic Press, New York, pp 91–119
11. ICHIHARA K 1984 *sn*-Glycerol-3-phosphate acyltransferase in a particulate fraction from maturing safflower seeds. Arch Biochem Biophys 232: 685–698
12. ICHIHARA K, T ASAHI, S FUJII 1987 1-Acyl-*sn*-glycerol-3-phosphate acyltransferase in maturing safflower seeds and its contribution to the non-random fatty acid distribution of triacylglycerol. Eur J Biochem 167: 339–347
13. RATTRAY JBM 1984 Biotechnology and the fats and oils industry-an overview. J Am Oil Chem Soc 61: 1701–1712
14. ROUGHAN PG, CR SLACK 1982 Cellular organization of glycerolipid metabolism. Annu Rev Plant Physiol 33: 97–132
15. SMITH RH, GL POWELL 1986 The critical micelle concentration of some physiologically important fatty acyl-coenzyme A's as a function of chain length. Arch Biochem Biophys 244: 357–360
16. STEFANSSON BR 1983 The development of improved rapeseed cultivars. *In* JKG Kramer, FD Sauer, WJ Pigden, eds, High and Low Erucic Acid Rapeseed Oils. Academic Press, Toronto, pp 143–159
17. STOBART AK, S STYMME 1985 The regulation of the fatty acid composition of the triacylglycerols in microsomal preparations from avocado mesocarp and the developing cotyledons of safflower. Planta 163: 119–125
18. STYMNE S, AK STOBART 1986 Triacylglycerol biosynthesis. *In* PK Stumpf, EE Conn, eds, The Biochemistry of Plants, Vol 9. Lipids. Academic Press, New York, pp 175–214
19. YAMASHITA S, K HOSAKA, Y MIKI, S NUMA 1981 Glycerolipid acyltransferases from rat liver: 1-acylglycerophosphate acyltransferase, 1-acylglycerophosphoryl-choline acyltransferase, and diacylglycerol acyltransferase. Methods Enzymol 71: 528–536