Control of fatty acid distribution in phosphatidylcholine of spinach leaves

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ABSTRACT The acylation of lysophosphatidylcholine by enzyme preparations from spinach leaves was studied. The acylation reaction was followed by the incorporation of ^{14}C-labeled fatty acids from the respective coenzyme A derivatives into phosphatidylcholine. The subcellular fraction with the highest specific activity was the microsomal fraction. Contaminating thioesterase activity which was encountered was inhibited by treatment with sodium dodecyl sulfate. The acyltransferase activity was only mildly inhibited by sulfhydryl reagents. Labeled fatty acid was primarily incorporated into phosphatidylcholine. When saturated and unsaturated fatty acyl CoA derivatives were used, the saturated derivatives were incorporated primarily into the 1-position of the glycerol moiety, and the unsaturated fatty acids went primarily to the 2-position. This pattern of incorporation agrees with the fatty acid distribution in vivo.

SUPPLEMENTARY KEY WORDS enzymic control · fatty acid distribution · fatty acyl coenzyme A

THE ACYLATION OF 1- or 2-acyl-*sn*-glycero-3-phosphorylcholine (1- or 2-acyl lyso PC) was first demonstrated in animal tissue in 1960, utilizing rat liver microsomes as the source of enzyme (1). The acylation of lyso PC has been shown in several other animal tissues and other organisms (2–6). Positional specificity for saturated and unsaturated fatty acids by this enzyme activity was shown (7), and this specificity could account for the fatty acid distribution in PC. In contrast to this acylation mechanism, the fatty acid distribution of PC in rat liver has been shown to be determined during de novo synthesis (8).

The acyltransferase reaction in plants has been studied only briefly. Bartels and van Deenen showed that when either ^{32}P-labeled lyso PC or ^{32}P-labeled lyso PE was added to a spinach homogenate with ATP, CoA, $MgSO_4$, and oleic acid, the disappearance of lysophosphatides correlated with an increase in PC and PE (9). This paper presents further studies on the acyltransferase reaction in spinach leaves, and attempts to elucidate the role of the acyltransferase in the control of the fatty acid distribution of PC in spinach leaves.

MATERIALS AND METHODS

Preparation of Subcellular Fractions

Spinach leaves were washed, deveined, and cut into small pieces prior to homogenization. The leaves were homogenized at 0°C in a Waring Blendor for 3–4 sec with 0.5 M sucrose, 10 mM Tris–HCl at pH 8.5 as homogenizing medium. The ratio of homogenizing medium to spinach leaves was 1.5:1 (v/w). The homogenate was filtered through four layers of cheesecloth. Subcellular fractions were prepared from the filtered homogenate by differential centrifugation.

The enzyme preparation used for most experiments was prepared by centrifuging the filtered homogenate at 20,000 *g* for 30 min. The pellet was discarded and the supernatant fraction was centrifuged at 100,000 *g* for 90 min. The microsomal pellet was then resuspended in 10 mM Tris–HCl pH 8.5, containing 0.5 mM DTT (Calbiochem, Los Angeles, Calif.) and 0.1% SDS (Matheson, Coleman & Bell, Los Angeles, Calif.). For the SDS treatment, the microsomal fraction from 200 *g* of deveined leaves usually was resuspended to give a protein concentration of 4–5 mg/ml, and kept for 30 min in the ice

Abbreviations: CoA, coenzyme A; DTT, dithiothreitol; lyso PC, lysophosphatidylcholine; lyso PE, lysophosphatidylethanolamine; lyso PG, lysophosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; POPOP, *p*-bis[2-(5-phenyloxazolyl)]-benzene; PPO, 2,5-diphenyloxazole; SDS, sodium dodecyl sulfate.

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bath. This preparation was then centrifuged for 90 min at 100,000 *g*. The pellet was discarded and the supernatant solution was either used immediately or was frozen and lyophilized. The lyophilized preparation retained approximately 80% of the acyltransferase activity for 2 wk. For utilization of the lyophilized enzyme, the preparation was dissolved in water at 0°C just prior to incubation with substrate.

Preparation of Fatty Acyl CoA Derivatives

Fatty acyl CoA derivatives of fatty acids were made by a modification of the method of Pande and Mead (10). The reaction mixture consisted of 100 μmoles of Tris–HCl, pH 7.4, 25 μmoles of NaF, 15 μmoles of ATP, 50 μg of CoA, 1 μmole of DTT, 4 μmoles of $MgSO_4$, 20–100 nmoles of ^{14}C-labeled fatty acid, and 2 mg of enzyme (9×10^6 *g*-min pellet) prepared from rat liver acetone powder (Sigma Chemical Co., St. Louis, Mo.) in a final volume of 1.00 ml. Incubation was for 2 hr at 37°C. The acyl CoA derivatives were isolated either by the method of Kornberg and Pricer (11) or by a modification (12). Radioactive purity of the resulting acyl CoA compounds was checked by chromatography on Silica Gel G (E. Merck A.G., Darmstadt, Germany) with hexane–diethyl ether–acetic acid 70:30:2 (v/v/v) as solvent. The areas corresponding to fatty acid were scraped off and counted in a scintillation counter (720 Series Liquid Scintillation System, Nuclear-Chicago Corp., Des Plaines, Ill.). The counts recovered in this fraction were compared to the total counts before thin-layer chromatography, and the radioactivity in the fatty acid fraction constituted less than 5% of the total counts. Purity was also checked by paper chromatography (11). If CoA was left out of the reaction mixture the synthesis of acyl CoA was reduced to less than 7% of that of reaction mixtures containing CoA. The purified acyl CoA derivatives were dissolved in water for use in enzyme assays. The maximum specific activities of ^{14}C-labeled fatty acids were used to calculate molar incorporation in the synthesis of PC (12).

The labeled fatty acids, palmitic acid-16-^{14}C, oleic acid-1-^{14}C, linoleic acid 1-^{14}C, linolenic acid-1-^{14}C, and stearic acid-U-^{14}C were obtained from Amersham/Searle Corp., Des Plaines, Ill. The labeled acyl CoA derivative myristoyl-1-^{14}C CoA was also purchased from Amersham/Searle, and palmitoyl-1-^{14}C CoA was purchased from New England Nuclear Corp., Boston, Mass.

Preparation of Lysophosphatidylcholine

Nonradioactive lyso PC was prepared by incubation of PC, isolated from either egg yolk or spinach chloroplasts, with *Crotalus adamanteus* venom (Sigma Chemical Co.) as described previously (13). The lyso PC was purified by thin-layer chromatography on Silica Gel G with chloroform–methanol–water 70:30:4 (v/v/v) as solvent. A portion of the lyso PC band was visualized by spraying the plate with 1% iodine in methanol; the portion that was not sprayed was scraped off, and the lyso PC was eluted with methanol. The lyso PC was dried under nitrogen and was dispersed in water. Phosphate was determined by the method of Bartlett (14).

Uniformly labeled 1-acyl-^{14}C lyso PC was prepared by digesting uniformly labeled PC-^{14}C isolated from *Chlorella vulgaris* lipid (Amersham/Searle) with *Crotalus adamanteus* venom phospholipase. The PC-^{14}C used constituted 98% of the radioactivity as judged by two-dimensional thin-layer chromatography (15). The resulting lyso PC-^{14}C was purified in the same manner as unlabeled lyso PC.

Incubation Mixtures

The incubation mixture for the incorporation of fatty acyl-^{14}C CoA into PC consisted of 80 μmoles of Tris–HCl, pH 7.0–8.5, acyl-^{14}C CoA with the addition or omission of 1-acyl lyso PC, and enzyme preparation in a final volume of 1.60 ml. The enzyme preparation was added last after a preincubation at 30°C for 5 min. Mixtures were incubated at 30°C for 1–5 min and reaction was terminated by the addition of 6 ml of methanol–chloroform 2:1. The lipid fraction was then extracted according to the method of Bligh and Dyer (16). The enzyme preparation used was either the SDS supernatant fraction as previously described or the resuspended microsomal fraction.

When incorporation of lyso PC-^{14}C into PC was measured, the incubation mixture was essentially the same as described for incorporation of fatty acid-^{14}C into PC. The concentration and purity of nonradioactive palmitoyl CoA were determined spectrophotometrically by making use of characteristic absorbances at 260 nm for the adenine moiety and 232 nm for the thioester. The palmitoyl CoA was obtained from Sigma Chemical Co.

For all incubation mixtures, controls without added enzyme preparation were run and were assayed for activity just as were the incubation mixtures containing enzyme preparations.

Analysis of Products

Incorporation of labeled fatty acyl groups into PC was determined routinely after chromatography on Silica Gel G thin-layer plates with chloroform–methanol–water 70:30:4 (v/v/v) as solvent, using egg PC as marker. Hydrolysis of labeled fatty acyl CoA to give labeled free fatty acid was routinely measured by chromatography on Silica Gel G thin-layer plates with hexane–diethyl ether–acetic acid 70:30:2 (v/v/v) as solvent, using stearic acid as marker (Calbiochem).

For determination of incorporation into various lipids, the chloroform layer was analyzed by two-dimensional

thin-layer chromatography (15) and with thin-layer chromatography using hexane–diethyl ether–acetic acid 70:30:2 (v/v/v) as solvent, with stearic acid, diglyceride (Sigma Chemical Co.), and triglyceride (Nutritional Biochemicals Corp., Cleveland, Ohio) as markers.

In all cases, spots were scraped and counted in a liquid scintillation counter using 10 ml of counting solution (0.5% PPO and 0.03% POPOP in toluene) and 0.5 ml of 1 M Hyamine hydroxide. Any quenching was corrected for by the channels-ratio method. Hyamine hydroxide, PPO, and POPOP were purchased from Amersham/Searle.

Positional analysis of incorporated radioactive fatty acids in PC was carried out by degradation of the isolated radioactive PC with *Crotalus adamanteus* venom phospholipase A (Sigma Chemical Co.), as described previously (13). Nonradioactive PC was added as marker. After digestion was complete, as judged by chromatography of an aliquot of the reaction mixture, the remainder of the sample was chromatographed on Silica Gel G thin-layer plates using hexane–diethyl ether–acetic acid 70:30:2 (v/v/v) as solvent. The areas corresponding to fatty acid and lyso PC were scraped and counted as previously described.

RESULTS

Subcellular Localization of Enzyme Activity

Tables 1 and 2 show the incorporation of palmitoyl-1-^{14}C CoA and linoleoyl-1-^{14}C CoA into PC by subcellular fractions of spinach leaves. The subcellular fraction with the highest activity on a protein basis is the microsomal fraction (100,000 *g* pellet) for both precursors. Most of the recovered activity in the palmitoyl-1-^{14}C CoA experiments was also in the microsomal fraction (total activity), but when linoleoyl-1-^{14}C CoA was used as substrate, most of the activity was in the 100,000 *g* supernatant. The same spinach leaf preparation was used for both substrates. Also, this same spinach leaf preparation was used for the determination of the subcellular distribution of CDP-choline-1,2-diglyceride-choline phosphotransferase (EC 2.7.8.2) (17). If the results with the three substrates are compared it is seen that the choline phosphotransferase activity is much more specifically associated with the microsomal fraction (17). It should be emphasized that the method of separation of subcellular fractions was not monitored by assay of marker enzymes and some cross contamination is probable.

The recovery of acyltransferase activity in the subcellular fractions for both acyl CoA derivatives was much lower than 100% of the acyltransferase activity of the unfractionated homogenate. This extremely low recovery of activity is probably not due to a lack of unlabeled substrate, since lyso PC was added in excess. The low recovery is most likely due to a competition for the acyl CoA substrate between the acyltransferase activity and thioesterase activity. In addition, perhaps there is some loss of activity due to inactivation of acyltransferase when the pellets were resuspended in 10 mM Tris–HCl buffer. The loss of activity could not be due to aging during the isolation of subcellular fractions, since all incubations were performed at the same time.

The recovery of thioesterase activity was rather different when palmitoyl CoA and linoleoyl CoA were used as substrates. This difference may have been due simply to variations in the homogenate activity used as a base for the calculations. It is notable that for both substrates the activities of the subcellular fractions on a protein basis are in good agreement, and the percentage distribution of the recovered activity in the two cases is also in very good agreement. This consistency is in contrast to the variations of distribution and activity of the acylating enzyme (Tables 1 and 2).

Purification of the Acyltransferase by Detergent Treatment

Although in Tables 1 and 2 the ratio of acyltransferase activity to thioesterase activity for both acyl CoA derivatives was quite high in the microsomal fraction, this ratio varied considerably with different microsomal preparations and was usually markedly lower than shown in Tables 1 and 2.

There is a tendency for a significant amount of the acyltransferase activity of spinach leaves to be found in the 100,000 *g* supernatant, but in order to solubilize acyltransferase activity further, and to avoid contamination with thioesterase, SDS was added in various concentrations to the microsomal preparation (Table 3). In addition to solubilizing the acyltransferase activity, treatment with the detergent increased the total acyltransferase activity and decreased the thioesterase activity.

The supernatant fraction obtained by treatment of the microsomal fraction with 0.05%–0.1% SDS was used for further studies because a relatively high specific activity was obtained and because the preparation could be lyophilized and redissolved easily for use in incubation mixtures (see Materials and Methods).

Time Course

The time course of incorporation of linolenoyl-1-^{14}C CoA into PC is shown in Fig. 1. When stimulation of incorporation of fatty acid into PC by addition of 1-acyl lyso PC was to be measured, the incubations were usually carried out for no longer than 2 min in order to observe maximal stimulation.

TABLE 1 INCORPORATION OF PALMITOYL-1-^{14}C CoA INTO PC AND FATTY ACID BY SUBCELLULAR FRACTIONS OF SPINACH LEAF

Fraction	Volume	Protein Concn.	Total Protein	Incorporation into PC		Incorporation into Fatty Acid	
	ml	*mg/ml*	*mg*	*pmoles/mg protein*	*% of recovered activity*	*pmoles/mg protein*	*% of recovered activity*
Homogenate	161	3.5	560	114	—	134	—
200 *g* pellet	7.1	3.6	26	12	2	144	7
1,000 *g* pellet	12.5	5.2	65	12	5	249	30
3,000 *g* pellet	4.0	2.5	10	95	6.5	160	3
20,000 *g* pellet	7.3	1.4	10	123	8.5	132	2.5
100,000 *g* pellet	5.0	5.7	29	236	46	23	1.5
100,000 *g* supernatant	143	3.3	470	10	32	64	56
Recovery of protein or enzymic activity	—	—	109%	—	23%	—	72%

Subcellular fractions were obtained as previously described (17). Reaction mixtures consisted of 80 μmoles of Tris–HCl, pH 3.0; 4 nmoles of spinach chloroplast lyso PC; 200 pmoles of palmitoyl-1-^{14}C CoA (20,000 dpm); and 0.2 mg of protein from each subcellular fraction, in a final volume of 1.60 ml. Incubation was for 2 min at 30°C.

TABLE 2 INCORPORATION OF LINOLEOYL-1-^{14}C CoA INTO PC AND FATTY ACID BY SUBCELLULAR FRACTIONS OF SPINACH LEAF

Fraction	Incorporation into PC		Incorporation into Fatty Acid	
	pmoles/mg protein	*% of recovered activity*	*pmoles/mg protein*	*% of recovered activity*
Homogenate	352	—	56	—
200 *g* pellet	233	10.5	100	5
1,000 *g* pellet	96	11	183	26
3,000 *g* pellet	324	5.5	107	2.5
20,000 *g* pellet	356	6	74	2
100,000 *g* pellet	519	25	16	1
100,000 *g* supernatant	53	42	62	63.5
Recovery of protein or enzymic activity	—	31%	—	146%

This experiment was done at the same time as that reported in Table 1. It is identical except for the replacement of palmitoyl CoA by 200 pmoles linoleoyl-1-^{14}C CoA (24,200 dpm).

pH Optima

The pH optima for the incorporation of acyl CoA derivatives into PC were found to be somewhat different. The pH optima were: palmitoyl CoA, 7.5–8.0; oleoyl CoA, 8.0; linoleoyl CoA, 7.0; and linolenoyl CoA, 7.5–8.0. The pH curve for incorporation of oleate into PC is shown in Fig. 2.

Inhibition of Acyltransferase Activity

Table 4 shows that the incorporation of oleoyl CoA and palmitoyl CoA into PC is not strongly affected by the addition of sulfhydryl binding agents. Therefore, the possibility that a sulfhydryl group is involved at the active site of the acyltransferase seems unlikely unless it is in a protected environment. This result is in agreement with the results of Lands and Hart (18) who used enzyme from animal sources, and it contrasts with the sulfhydryl sensitivity in the de novo synthesis (17).

TABLE 3 SOLUBILIZATION OF TRANSACYLASE ACTIVITY BY VARIOUS CONCENTRATIONS OF SODIUM DODECYL SULFATE

Detergent Concn.	Microsomal Pellet			Microsomal Supernatant			Sum of Incorporations of Pellet and Supernatant	
	Protein Concn.	PC	Fatty Acid	Protein Concn.	PC	Fatty Acid	PC	Fatty Acid
%	*mg/ml*	*dpm*		*mg/ml*	*dpm*		*dpm*	
0.00	—	5730	720	—	1990	2840	7720	3560
0.01	3.0	6130	750	1.0	2350	2690	8480	3440
0.02	2.6	5570	630	1.0	3670	1640	9240	2270
0.05	2.3	5970	630	1.6	5410	1150	11,380	1780
0.10	2.0	5610	620	2.4	6930	860	12,540	1480
0.20	1.2	4090	640	3.5	570	1040	4660	1680

Incubation mixture: 60 μmoles of Tris–HCl, pH 8.5; 350 pmoles of oleoyl-1-^{14}C CoA (13,500 cpm); 11.5 nmoles of spinach chloroplast lysophosphatidylcholine; and 0.1 ml of microsomal pellet or microsomal supernatant. Incubation volume was 1.60 ml. Incubation was for 30 min at 30°C. Incubation with detergent and the centrifugation were done as described in Materials and Methods. The pellets after centrifugation were resuspended in 2.0 ml of a medium containing 0.095 M Tris–HCl, pH 8.5, and 0.5 mM DTT.

TABLE 4 INHIBITION OF ACYLTRANSFERASE ACTIVITY

Expt.	Acyl CoA	Inhibitor Concentration	Incorporation into PC	
			dpm	%
1	Oleoyl CoA	None	2244	100
	Oleoyl CoA	1 mM iodoacetamide	2484	111
	Oleoyl CoA	1 mM *N*-ethylmaleimide	2544	114
	Oleoyl CoA	1 mM *p*-hydroxymercuribenzoate	1564	70
2	Palmitoyl CoA	None	1980	100
	Palmitoyl CoA	10 mM iodoacetamide	1482	75
	Palmitoyl CoA	10 mM *N*-ethylmaleimide	816	41
	Palmitoyl CoA	0.94 mM *p*-hydroxymercuribenzoate	1148	58
	Palmitoyl CoA	9.4 mM *p*-hydroxymercuribenzoate	682	34

Incubation mixture: 75 μmoles of Tris–HCl, pH 8.5; 5 nmoles of spinach chloroplast lyso PC; and 350 pmoles of oleoyl-1-^{14}C CoA (13,500 dpm), or 125 pmoles of palmitoyl-16-^{14}C CoA (10,400 dpm). A 0–10% $(NH_4)_2SO_4$ precipitate of a 20,000 *g* × 30 min supernatant of spinach homogenate was prepared by stirring the supernatant with $(NH_4)_2SO_4$ for 30 min at 0°C. The precipitate was sedimented by centrifugation at 20,000 *g* for 20 min, and the pellet was resuspended in 0.01 M Tris–HCl, pH 8.0. Samples of this preparation were used as enzyme source; 180 μg of protein was added. This fraction is equivalent to microsomes. Preincubation without radioactive substrate added was for 10 min at 30°C, and incubation was for 30 min at 30°C. Final incubation volume was 1.60 ml.

Distribution of Incorporated Fatty Acid

Table 5 shows the distribution of linoleoyl-1-^{14}C CoA incorporated into various lipid fractions. The majority of the fatty acid was incorporated into PC, with smaller amounts incorporated into diglyceride, an unknown fraction, and PE. The results indicate that either there is little or no transferase activity in the enzyme preparation for PG and PE or else there is little or no lyso PG or lyso PE present.

Lyso PC Stimulation of Incorporation

Table 6 shows the effect of added 1-acyl lyso PC on the incorporation of acyl CoA derivatives into PC. In all cases there was sufficient endogenous acceptor to provide a measurable rate of acylation, but in all cases added lyso PC stimulated incorporation.

Table 7 shows the incorporation of 1-acyl lyso PC-U-^{14}C into PC. The addition of unlabeled palmitoyl CoA increased the incorporation fivefold. This indicated that the incorporation of labeled acyl CoA derivatives was not an exchange reaction.

Positional Specificity of the Acylation Reaction

A comparison of the acylation reaction for different acyl CoAs depended on the choice of acyl CoA concentrations that would not be inhibitory. Concentration curves were run for palmitoyl CoA and linoleoyl CoA, and these are shown in Fig. 3. There was some suggestion of inhibition

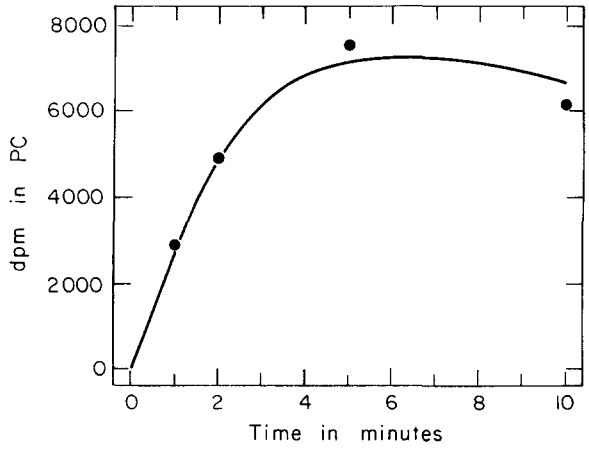

FIG. 1. Time course of incorporation of linolenoyl-1-^{14}C CoA into PC. Incubation mixture: 80 μmoles of Tris–HCl, pH 8.5; 2.5 nmoles of spinach chloroplast lyso PC; 180 pmoles of linolenoyl-1-^{14}C CoA (17,900 dpm); and 0.1% SDS supernatant (50 μg of protein). Incubation volume was 1.60 ml. Incubation was at 30°C.

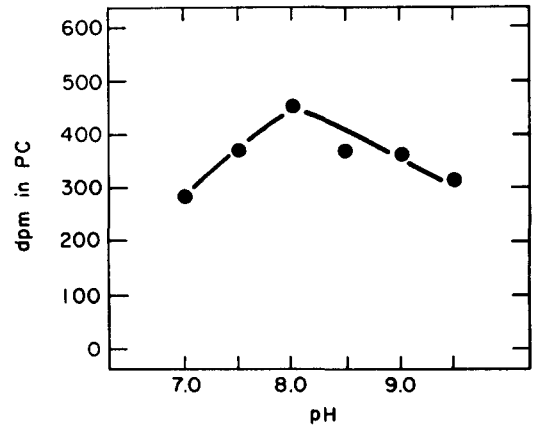

FIG. 2. Dependence of acyltransferase activity on pH. Incubation mixture: 80 μmoles of Tris–HCl, varying pH; 170 pmoles of oleoyl-1-^{14}C CoA (3280 dpm); 2.5 nmoles of spinach chloroplast lyso PC; and 0.1% SDS supernatant of microsomes containing 50 μg of protein. Incubation was for 1 min at 30°C. Incubation volume was 1.60 ml.

TABLE 5 DISTRIBUTION OF INCORPORATED RADIOACTIVITY FROM LINOLEOYL-1-^{14}C CoA

Lipid Fraction	Incorporation	
	dpm	%
Diglyceride	164	10.5
Fatty acid	0	—
Triglyceride	0	—
Sulfolipid	0	—
Digalactosyl diglyceride	5	0.3
Phosphatidylcholine	1270	83
Unidentified	66	4
Phosphatidylglycerol	4	0.2
Phosphatidylethanolamine	30	2
Monogalactosyl diglyceride	0	—

Incubation mixture: 80 μmoles of Tris–HCl, pH 7.0; 96 pmoles of linoleoyl-1-^{14}C CoA (11,200 dpm); and 0.01 ml of 0.1% SDS supernatant (50 μg of protein). Incubation was for 1 min at 30°C. Incubation volume was 1.60 ml. SDS supernatant was prepared as described in Materials and Methods. The various lipid fractions were obtained by chromatography on thin-layer plates, using hexane–diethyl ether–acetic acid 70:30:2 and a two-dimensional system (15) as solvent systems.

TABLE 6 STIMULATION OF INCORPORATION OF ACYL CoA INTO PHOSPHATIDYLCHOLINE BY ADDED LYSOPHOSPHATIDYLCHOLINE

Expt.	Acyl CoA	− Lyso PC	+ Lyso PC	Increase
		dpm		%
1	Oleoyl CoA	162	345	113
2	Linoleoyl CoA	1270	2640	108
3	Linolenoyl CoA	693	1094	58

Incubation mixtures: (1) 200 pmoles of oleoyl-1-^{14}C CoA (13,500 dpm); 0.01 ml of 0.10% SDS supernatant (50 μg of protein); 80 μmoles of Tris–HCl, pH 8.5; and 2.5 nmoles of spinach lyso PC. Incubation was for 1 min at 30°C. (2) 96 pmoles of linoleoyl-1-^{14}C CoA (11,200 dpm); 0.01 ml of 0.10% SDS supernatant (50 μg of protein); 80 μmoles of Tris–HCl, pH 7.0; and 2.5 nmoles of spinach lyso PC. Incubation was for 1 min at 30°C. (3) 100 pmoles of linolenoyl-1-^{14}C CoA (18,200 dpm); 0.01 ml of 0.10% SDS supernatant (50 μg of protein); 80 μmoles of Tris–HCl, pH 7.5; and 2.0 nmoles of spinach lyso PC. Incubation was for 2 min at 30°C. All incubation volumes were 1.60 ml.

at the higher concentrations of palmitoyl CoA. The concentration of acyl CoA used in studies of acylation as a function of the acyl substituent was 12.5 × 10^{-8} M; this was the beginning of the "saturation" part of the concentration curves for the two acyl CoAs.

Table 8 shows the positional distribution of the fatty acids incorporated into PC. The general pattern of incorporation fits the distribution pattern in vivo (13), that is, the saturated fatty acids tend to be more in the 1-position of glycerol than do the unsaturated fatty acids. The incorporation into the 1-position of glycerol is probably due to the presence of endogenous 2-acyl lyso PC, but it is not clear whether this has arisen by action of an endogenous phospholipase A or is due to the fact that an equilibrium between 1-acyl lyso PC and 2-acyl lyso PC is reached (18).

TABLE 7 INCORPORATION OF RADIOACTIVE LYSOPHOSPHATIDYLCHOLINE INTO PHOSPHATIDYLCHOLINE

+ Palmitoyl CoA	− Palmitoyl CoA
dpm	*dpm*
117	29
131	24

Incubation mixture: 80 μmoles of Tris–HCl, pH 8.0; lyso PC-U-^{14}C (4100 dpm); 0.05 ml of 0.06% SDS supernatant (40 μg of protein); and 10 nmoles of palmitoyl CoA. Incubation was for 30 min at 30°C. Incubation volume was 1.60 ml.

The most important point to be made from the data in Table 8 is the positional distribution of the incorporated acyl chains. The absolute activities of acyltransferases for different acyl groups may be differentially affected by the SDS treatment. In both Fig. 3 and Table 8 (A) the transferase activity for palmitate is low in comparison with that for linoleate, but in Table 8 (B), where the untreated microsomes were used as enzyme source, the activity with respect to palmitate is relatively high.

DISCUSSION

The acyltransferase activity of spinach leaf microsomes shows a specificity of acylation similar to that of animals (18). The lack of specificity in the choline phosphotransferase step of de novo synthesis of phosphatidylcholine (17) indicates that the acyltransferase activity may be responsible for the distribution of fatty acids in phosphatidylcholine of spinach leaves. This mechanism of control of fatty acid distribution contrasts with that for galactolipids of spinach, where specificity for the diglyceride acceptor is observed (19), but it is similar to the

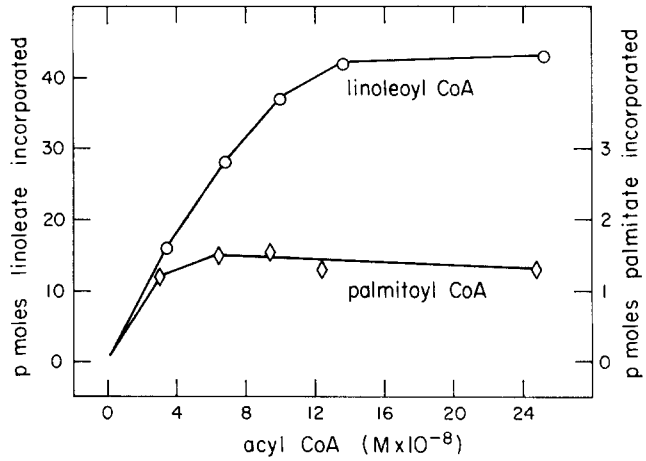

FIG. 3. Acyl CoA concentration curves for transferase activity. Incubation mixture: 80 μmoles of Tris–HCl, pH 8.5; 2 nmoles of spinach lyso PC; 0.1% SDS supernatant of microsomes containing 50 μg of protein; and varying amounts of radioactive linoleoyl CoA or palmitoyl CoA as indicated. Final volume was 1.60 ml. Incubation was for 1 min at 30°C.

TABLE 8 SPECIFICITY OF ACYL CoA INCORPORATION INTO PHOSPHATIDYLCHOLINE

Acyl CoA	Incorporation		% Removed by Phospholipase A
	dpm	*pmoles*	
A. 200 μg of protein: supernatant of 0.08% SDS supernatant from microsomes			
Myristoyl-1-^{14}C CoA	454	49	13
Palmitoyl-1-^{14}C CoA	169	1.7	28
Stearoyl-U-^{14}C CoA	472	2.2	40
Oleoyl-1-^{14}C CoA	1280	67	90
Linoleoyl-1-^{14}C CoA	7239	62	93
Linolenoyl-1-^{14}C CoA	5157	52	95
B. 200 μg of protein: untreated spinach leaf microsomes			
Myristoyl-1-^{14}C CoA	680	72	53
Palmitoyl-1-^{14}C CoA	2516	26	47
Oleoyl-1-^{14}C CoA	1452	74	91
Linoleoyl-1-^{14}C CoA	3088	26	91
Linolenoyl-1-^{14}C CoA	1466	14	84

Incubation mixture: 80 μmoles of Tris–HCl, pH 8.0; 200 μg of enzyme protein; 4 nmoles of spinach chloroplast lyso PC. 200 pmoles of each acyl CoA was added. Myristoyl-1-^{14}C CoA, 2100 dpm; palmitoyl-1-^{14}C CoA, 20,000 dpm; stearoyl-U-^{14}C CoA, 42,600 dpm; linoleoyl-1-^{14}C CoA, 23,400 dpm; linolenoyl-1-^{14}C CoA, 19,800 dpm; oleoyl-1-^{14}C CoA, 3840 dpm. Final reaction volume was 1.60 ml. Incubation was for 2 min at 30°C.

enzyme specificity in the choline phosphotransferase reaction in rat liver (20).

However, recent studies with plant tissues indicate another mechanism for control of fatty acid distribution and a mechanism for the synthesis of polyunsaturated fatty acids in plant tissue (21, 22). In both *Chlorella vulgaris* (21) and pumpkin leaves (22), PC is believed to be a necessary cofactor in the biosynthesis of polyunsaturated fatty acids. If there is such a role for PC in spinach leaves, it is puzzling why fatty acid synthesis takes place in the chloroplasts (23) and PC biosynthesis takes place in the microsomes (17), unless there were a pool of phosphatidylcholine in the chloroplasts (13) which could serve as a cofactor for polyunsaturated fatty acid synthesis and a transport mechanism for fatty acid or phosphatidylcholine (24, 25). In either case, the acyltransferase activity could play a role in placing a suitable precursor fatty acid in the proper position on phosphatidylcholine. Also, since the de novo synthesis of phosphatidylcholine takes place primarily in the microsomal fraction in spinach leaves (17) and the fatty acid compositions of chloroplast and microsomal phosphatidylcholines differ (13), the acyltransferase found in chloroplasts may modify the composition of phosphatidylcholine transported from the microsomes.

Various difficulties are encountered in the use of acyl CoAs as substrates. They are frequently inhibitory at higher concentrations, but this inhibition can be prevented by the inclusion of excess protein in the reaction mixture (26). Another problem associated with the use of acyl CoAs is a consequence of the low critical micellar concentrations (27). Substrate concentration curves which extend beyond the critical micellar concentrations should be carefully interpreted; for example, Barden and Cleland (27) have observed that acylation of glycerol-1-phosphate appears to prefer the monomeric acyl CoA, but the competing hydrolase appears to prefer the micellar form. The work reported in this paper was always conducted below the critical micellar concentrations of the acyl CoAs, and substrate concentration curves showed that the concentrations used were not inhibitory.

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