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Acyltransferase-catalyzed Synthesis of Lecithin in Rat Lung and Liver Microsomes

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Introduction

It has been known that lung tissues contain a large amount of dipalmitoyl lecithin as a pulmonary surfactant.

Previous works[1),2)] in our laboratory revealed a relatively high incorporation of labeled palmitic acid or palmitoyl-GPC into a saturated species of lecithin in rat lung slices in comparison with other species and it was in part elucidated with the involvement of the enzyme catalyzing the acyltransfer between two molecules of lysolecithin[3~5)]. However, other investigators[6),7)] suggested that the acylCoA : lysolecithin acyltransferase has an important role on synthesizing the saturated species of lecithin in lung.

In the present paper, the acylation of lysolecithin with various fatty acids was investigated both in lung and liver microsomes.

Materials and Methods

Chemicals

(9, 10-$^3H_2$) palmitic acid and (1-^{14}C) linoleic acid were purchased from The Radiochemical Centre, Amersham, Bucks., U. K. ATP disodium salt, coenzyme A, oleic acid, linoleic acid and arachidonic acid were bought from Sigma Chemical Co., St. Louis, Mo., U.S.A.

Preparation of microsomes

Wistar rats, weighing about 200 g were killed by exsanguination. Microsomal fraction was prepared from lung and liver tissues according to the procedures described by Van den Bosch *et al.*[8)] Microsomal pellet was rinsed twice with 0.125 MKCl-0.1 MTris pH 7.4 and suspended in the same buffer at a concentration of 2 mg protein per mℓ. The suspension was divided into small portions and stored at $-20°C$ until use.

Preparation of substrates

Non-labeled lysolecithin was prepared from egg lecithin by hydrolysis with *Crotalus adamanteus* venom. 1-(9, 10-$^3H_2$) palmitoyl-GPC was prepared by hydrolysis of 1-(9, 10-$^3H_2$) palmitoyl lecithin which was synthesized from beef heart plasmalogenic lecithin according to the procedure of Robertson and Lands[9)]. Lysolecithins were purified by TLC and stored at $-20°C$ in chloroform. An aliquot of the chloroform solution was evaporated in a nitrocellulose tube under a stream of N_2, buffer solution was poured

Abbreviations
GPC, glycerylphosphoryl choline
TLC, thin-layer chromatography

into the tube and sonicated at 1A for 2 min in a Kubota Insonator Model 200 M to make a working solution of lysolecithin at 0.2 mM.

Fatty acid mixtures were prepared from rat liver lecithin after mild alkaline hydrolysis. The fatty acid composition was as follows; 14 : 0, 8.1% ; 16 : 0, 21.7% ; 18 : 0, 24.2% ; 18 : 1, 8.7% ; 18 : 2, 14.1% and 20 : 4, 23.1%. Emulsion of fatty acid was prepared by sonication in Tris-HCl buffer pH 7.4 at a concentration of 0.5 mM just before use.

Determination of the acyltransferase reaction

The incubation mixture contained, unless otherwise stated, 80 μmoles of Tris-HCl pH 7.4, 95 nmoles of 1-(9, 10-$^3H_2$) palmitoyl-GPC, 200 nmoles of fatty acids, 10 μmoles of $MgCl_2$, 10 μmoles of ATP, 200 nmoles of CoA and 0.5 mg of microsomal protein in a total volume of 1.0 mℓ.

In experiments where the labeled fatty acids were used, the mixture contained 80 μmoles of Tris-HCl pH 7.4, 190 nmoles of 1-acyl-GPC, 80 nmoles of (9, 10-$^3H_2$) palmitic acid and 80 nmoles of (1-^{14}C) linoleic acid, 10 μmoles of $MgCl_2$, 10 μmoles of ATP, 200 nmoles of CoA and 0.2 mg of microsomal protein in a final volume of 1.0 mℓ. After the indicated periods of incubation at 37°C in a shaking water bath, the reaction was stopped by the addition of 2 vol. of chloroform-methanol (1 : 4, v/v) and the lipids were extracted by successive additions of 6 vol. of chloroform-methanol (4 : 1, v/v) and 0.6 vol. of water. Lecithin, lysolecithin and free fatty acids were isolated by TLC on Kiesel gel G. Lecithin was fractionated into various molecular species after conversion into 1, 2-diglyceride with phospholipase C from *Cl. Welchii*, followed by TLC on $AgNO_3$-impregnated plates.

Radioactive spots were scraped into counting vials containing 10 mℓ of the dioxane scintillation fluid described by Snyder[10]. The radioactivity was measured by Horiba liquid scintillation spectrometer model LS-500. Reaction rate was estimated from the percentage of the radioactivity transferred into lecithin.

In experiments in which ^{3}H- and ^{14}C-labeled fatty acids were used or subfractionation of lecithin were carried out, the lecithins were eluted from the silica with a solvent described by Arvidson[11]. After evaporation of the solvent, 10 mℓ of toluene scintillation fluid was added and counted.

Protein was determined by the method of Lowry *et al.*[12] and phosphorus was by Bartlett's method[13]. Free fatty acid was determined colorimetrically according to the method of Itaya and Ui[14].

Results and Discussion

Time courses of the acylation of 1-(^{3}H) palmitoyl-GPC in lung and liver microsomes were shown in Fig. 1. When ATP and CoA were omitted, no acylation was observed. However, a considerable formation of lecithin occurred in the absence of the exogenous fatty acids suggesting the acylation using endogenous fatty acids took place. (c.f. Table 1). The contents of free fatty acids in lung and liver microsomes were 35 nmoles and 55 nmoles per mg of protein, respectively. The effect of protein concentration was studied under the addition of free fatty acids (Fig. 2). The rate of acylation in lung was far below the rate in liver microsomes. It was noted that the acylation of

lysolecithin in liver microsomes was affected significantly with the composition of the exogenous fatty acid added in the medium, while in lung it was constant even if the compositions of the surrounding fatty acids were varied (Table 1). Greater the amount of palmitic acid in the mixture, lower the acylation of lysolecithin was in liver.

The distribution of isotope in various molecular species of lecithin formed was studied by $AgNO_3$-impreganted TLC after the incubation for 15 min (Table 2). The saturated-monoenoic fractions in lung had the greatest radioactivity among other species except when arachidonic acid was singly added in the incubation medium. In the latter case, 1-(^{3}H) palmitoyl-GPC was predominantly acylated into tetraenoic fraction. The preference of arachidonic acid for the acyltransferase was more evident in liver microsomes coincided with the data reported by other investigators[15~17]. However, the acylation of arachidonic acid in lung microsomes was less preferencial as compared with palmitic acid. A significant uptake of palmitic acid into 2-positions of lecithin in lung by acyl-CoA : lysolecithin acyltransferase was also reported by Frosorono *et al.*[6] and Vereyken *et al.*[7]. A considerable formation of saturated-monoenoic lecithin observed in the absence of external fatty acids might be accounted for a higher distribution of palmitic acid among the endogenous free fatty acids (Table 3).

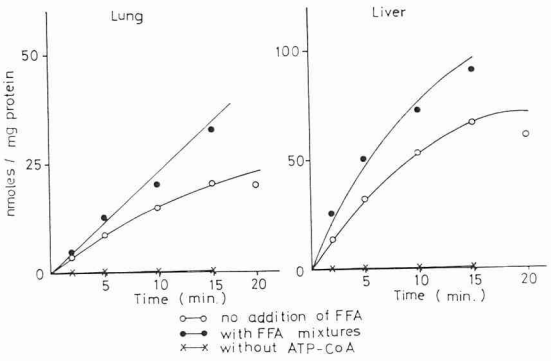

Fig. 1 Time courses of lecithin formation from 1-(9, 10-$^3H_2$) palmitroyl GPC

Incubation mixture contained 80 μmoles of Tris-HCl pH 7.4, 10 μmoles of ATP, 20 nmoles of CoA, 10 μmoles of $MgCl_2$, 70 nmoles of 1-(9, 10-$^3H_2$) palmitoyl GPC, 200 nmoles of fatty acid mixture and 0.25 mg of microsomal protein in a final volume of 1.0 mℓ. At each period of incubation, an aliquot (0.2 mℓ) was pipetted off and lipids were extracted as described in the text.

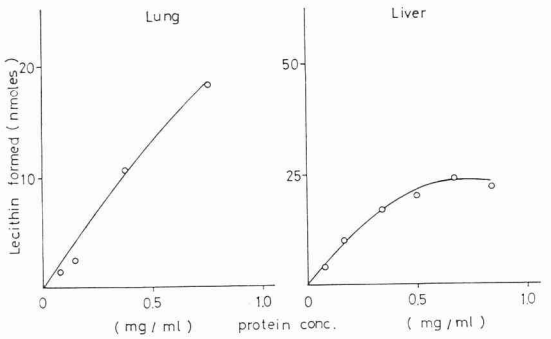

Fig. 2 Effect of protein concentration on the acylation of 1-(9, 10-$^3H_2$) palmitoyl GPC

Incubation conditions were the same as described in a legend of Fig. 1 except the concentration of microsomal protein were varied. Incubation was carried out at 37°C for 15 min.

Time courses of the acylation of 1-acyl GPC with (^{3}H) palmitic acid and (^{14}C) linoleic acid were presented in Fig. 3. Although a higher incorporation of linoleic acid into lecithin in lung was observed, the amount of the lecithin acylated with linoleic acid was about one-third of that acylated in liver microsomes. As regards palmitic acid incorporation, nearly the same amount of lecithin was formed in lung as well as in liver. The similar data were reported by Vereyken *et al.*[7]. It was suggested from the

Table 1 *Acylation of 1-[9, 10-$^3H_2$] palmitoyl-sn-glycero-3-phosphorylcholine in the presence of lung and liver microsomes from rat*

Fatty acids added (μM)		Lung	Liver
		n moles/mg protein/15 min	
None		20.9 ± 5.23 (5)	53.2 ± 16.41 (4)
Mixture*	200	23.6 ± 3.98 (5)	80.7 ± 8.91 (5)
20 : 4	200	25.1 ± 0.75 (3)	73.3, 62.4
20 : 4 16 : 0	150 50	23.9 ± 3.08 (3)	34.8
20 : 4 16 : 0	100 100	24.3 ± 1.70 (3)	22.9, 21.4
20 : 4 16 : 0	50 150	21.8 ± 2.70 (3)	15.9
16 : 0	200	24.7 ± 2.85 (3)	8.7, 5.6

mean value ± stardard deviation

* The fatty acid mixture was obtained from rat liver lecithin after mild alkaline hydrolysis. The fatty acid composition was as follows;
14 : 0, 8.1% 16 : 0, 21.7% 18 : 0, 24.2% 18 : 1, 8.7% 18 : 2, 14.1% 20 : 4, 23.1%
Figures in parentheses show the numbers of experiments.
The experimental conditions were described in the text.

Table 2 *Isotope distribution (%) into various molecular species of lecithin after incubation of microsomes with 1-[9, 10-$^3H_2$] palmitoyl-sn-glycero-3- phosphorylcholine*

Fatty acids added (μM)		Numbers of expt.	Lung S	M	D	T	H	Numbers of expt.	Liver S	M	D	T	H
None			48.5	17.8	18.5	14.9			30.7	11.1	19.6	38.5	
			83.2		10.4	6.9	1.3		42.8		20.9	24.0	7.9
Mixture*	200		33.5	21.6	20.9	16.5	7.4		13.3	5.9	34.8	44.3	1.6
		8	49.4 ± 4.55		18.0 ± 4.43	25.3 ± 4.93	5.7 ± 1.83	9	21.1 ± 6.56		46.8 ± 6.16	26.0 ± 6.34	4.7 ± 0.46
20 : 4	200	3	40.8 ± 0.37		7.0 ± 0.70	48.4 ± 1.04	3.2 ± 0.76	2	9.2 ± 2.9		12.1 ± 5.9	74.0 ± 8.4	4.5 ± 0.3
20 : 4 16 : 0	150 50	3	73.2 ± 6.41		4.9 ± 1.25	20.2 ± 5.50	1.5 ± 0.96		32.1		18.0	41.6	8.3
20 : 4 16 : 0	100 100	3	82.9 ± 4.39		3.9 ± 1.13	11.7 ± 3.74	1.1 ± 0.57	2	36.6 ± 10.0		16.5 ± 3.4	41.1 ± 11.0	5.5 ± 1.9
20 : 4 16 : 0	50 150	3	84.1 ± 6.26		3.9 ± 1.13	11.0 ± 5.49	0.8 ± 0.64		42.3		18.6	30.9	8.3
16 : 0	200	3	81.8 ± 7.34		8.3 ± 3.24	7.9 ± 2.23	1.2 ± 0.36	2	43.2 ± 1.3		22.3 ± 2.1	28.3 ± 2.1	5.5 ± 2.0

* Fatty acid composition was same as in Table 1.
S : saturated M : monoenoic D : dienoic T : tetraenoic H : hexaenoic species

Table 3 *Composition of the endogenous free fatty acids in lung and liver microsomes*

Fatty acids	14 : 0	16 : 0	18 : 0	18 : 1	18 : 2	20 : 4
		mole %				
Lung Ms	8.0	56.6	10.6	17.7	7.0	trace
Liver Ms	trace	45.6	19.8	14.6	13.6	6.4

Microsomal lipids were extracted by the procedure described in the text. Free fatty acids were isolated by TLC and mole percent was estimated from the data obtained by gas-liquid chromatographic analysis.

present data that the lung acyltransferase was capable of selecting palmitic acid for the acylation of 1-acyl GPC among other fatty acids in spite of the appreciable existence of unsaturated fatty acids which was primarily used for the acylation in liver microsomes. However, the rate of acylation seemed to be so low to elucidate a high content of dipalmitoyl lecithin in lung.

The acyltransfer reaction observed in the present work was catalyzed with multiple enzyme systems such as fatty acyl CoA synthetase and acyltransferase. It has been reported by other workers[13,19] that fatty acyl CoA synthetase in liver particles was not a rate limiting step for the incorporation of fatty acids into lecithin molecules. More precise investigation using several acylCoA would be necessary for clarifying the role of acyl CoA : lysolecithin acyltransferase on the synthesis of dipalmitoyl-lecithin in lung.

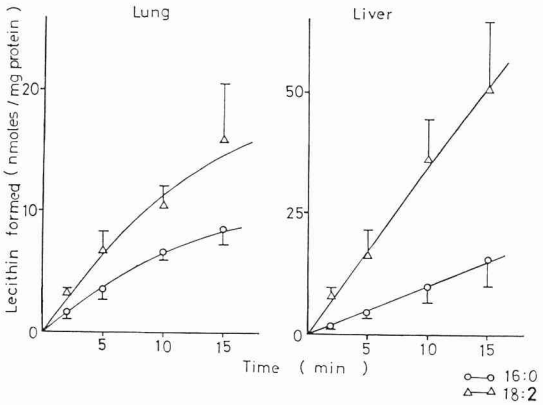

Fig. 3 Acylation of 1-acyl GPC with labeled fatty acids.

Incubation mixture contained 80 μ moles of Tris-HCl pH 7.4, 10 μ moles of ATP, 200 nmoles of CoA, 10 μ moles of $MgCl_2$, 190 nmoles of 1-acyl GPC, 80 nmoles of both (9, 10-$^3H_2$) palmitic acid and (1-^{14}C) linoleic acid and 0.17-0.25 mg of microsomal protein in a final volume of 1.0 mℓ. At each period of incubation, aliquots (0.25 mℓ) were pipetted off and lipids were extracted as described in the text. A half range of the standard deviation obtained from eight series of experiments was also represented.

Summary

The acylation of lysolecithin with various fatty acids was investigated in rat lung and liver microsomes.

In lung microsomes, the rate of the synthesis of lecithin from 1-(9, 10-$^3H_2$) palmitoyl-GPC and fatty acid mixtures was slow but constant even when the compositions of the exogenous fatty acids were varied. Palmitic acid was appreciably incorporated into a saturated species of lecithin.

In contrast, in liver microsomes, arachidonic acid was primarily incorporated into tetraenoic species of lecithin and the rate of acylation was gradually decreased with

the increase in palmitic acid content in the incubation medium. It seems unlikely that a large amount of dipalmitoyl lecithin in lung was mainly synthesized by the acylCoA : lysolecithin acyltransferase pathway.

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