SHORT COMMUNICATIONS

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The conversion of unsaturated 2-acyl-*sn*-glycero-3-phosphorylcholines into major molecular species of phosphatidylcholines

The transfer of unsaturated fatty acids from their CoA derivatives to phosphatidylcholine as catalysed by rat liver microsomes is preferentially stimulated by the addition of 1-acyl-*sn*-glycero-3-phosphorylcholine (1-acyl-3-*sn*-GPC)[1-4]. Conversely, 2-acyl-*sn*-glycero-3-phosphorylcholine (2-acyl-3-*sn*-GPC) preferentially enhances the incorporation of saturated acyl chains[1,2,5].

In this paper acylation experiments are described using 1-[9,10-$^{3}H_2$]stearoyl-3-*sn*-GPC in combination with either 2-[1-^{14}C]oleoyl- or 2-[1-^{14}C]linoleoyl-3-*sn*-GPC in order to be able to investigate the fate of these acyl chains during the conversion of the monoacyl-3-*sn*-GPC's into the major molecular species of phosphatidylcholine.

1-[9,10-$^{3}H_2$]Stearoyl-3-*sn*-GPC was synthesized as described earlier[5]. The purified compound showed a specific activity of $6.5 \cdot 10^9$ disint./min per mmole.

Unsaturated 2-acyl-3-*sn*-GPC's were prepared by making use of the finding[6] that highly purified lipases hydrolyse exclusively the 1-acyl–ester linkage of phospholipids. 1-Acyl-2-[1-^{14}C]oleoyl(or linoleoyl)-3-*sn*-GPC were biochemically synthesized[7] in incubation mixtures containing: 50 μC of [1-^{14}C]oleic(or linoleic) acid, 1 μmole of 1-stearoyl-3-*sn*-GPC, 50 μmoles of $MgCl_2$, 50 μmoles of ATP, 1 μmole of CoA, 60 μmoles of Tris buffer (pH 7.2) and 50 mg of rat liver microsomal protein in a final volume of 3.0 ml of 0.125 M KCl. After 1-h incubation at 37° the labelled lecithins were extracted and purified according to established procedures.

For the preparation (method worked out by SLOTBOOM[8]) of 2-[1-^{14}C]oleoyl(or linoleoyl)-3-*sn*-GPC, 3 mg of the labelled lecithins were mixed in solution with 12 mg of synthetic dioleoyl (or linoleoyl) lecithin. After evaporation of the solvent, 3 mg of sodium deoxycholate, 10 μg of albumin and 1 ml of a 0.1 M calcium borate buffer (pH 6.5; [Ca^{2+}], 5 mM) were added. The mixture was briefly sonicated and shaken vigorously at 30° with 2 mg of *Rhizopus arrhizus* lipase until the reaction was complete as could be deduced from thin-layer chromatograms on microscopic slides. Purification of the 2-acyl-3-*sn*-GPC was done by chromatography of the lipid extract of the incubation mixture on a Sephadex LH20 column with chloroform–methanol (1:1, v/v) as elution solvent. Further details of this procedure will be published elsewhere[9]. Typical preparations of the purified monoacyl phosphatidylcholines had the following specific activities: 2-[1-^{14}C]oleoyl-3-*sn*-GPC, $1.1 \cdot 10^9$ distint./min per mmole; and 2-[1-^{14}C]linoleoyl-3-*sn*-GPC, $4.5 \cdot 10^8$ disint./min per mmole.

The structure of the monoacyl-3-*sn*-GPC was checked by means of phospholipase A degradation. With both substrates 88% of the fatty acids were released, whereas nonenzymatic hydrolysis was limited to less than 2%. Fatty acid analysis on the 2-[1-^{14}C]oleoyl(or linoleoyl)-3-*sn*-GPC showed that 95% and 89% of the ^{14}C was recovered in the monoenoic and dienoic fractions, respectively.

Acylation studies with the isomeric lysolecithins showed a much higher acyla-

Abbreviations: 1-(or 2)-acyl-3-*sn*-GPC, 1-(or 2)-acyl-*sn*-glycero-3-phosphorylcholine.

tion capacity per mg of protein for the microsomal preparations when compared with the mitochondrial fractions (Fig. 1). Further experiments, carried out with microsomes from rat liver, indicated the use of unsaturated 2-acyl-3-*sn*-GPC which equals or even surpasses that of saturated 1-acyl-3-*sn*-GPC. The relative amounts of 1-acyl- and 2-acyl-3-*sn* GPC's converted into lecithin are influenced by added fatty acids to

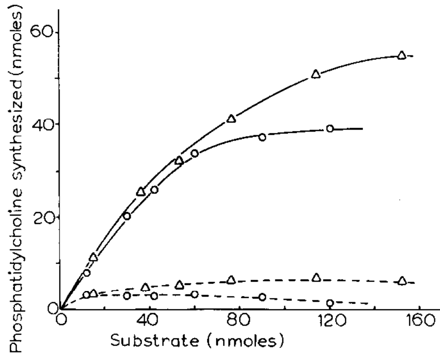

Fig. 1. Influence of substrate concentration on the acylation of monoacyl phosphatidylcholines by mitochondrial and microsomal preparations of rat liver. The incubation mixture consisted of the indicated amounts of substrate, 40 nmoles of each palmitate and linoleate, 10 μmoles of $MgCl_2$, 10 μmoles of ATP, 0.2 μmole of CoA, 60 μmoles of Tris buffer (pH 7.2) and 0.4 mg of mitochondrial or 0.2 mg of microsomal protein in a final volume of 0.9 ml. Incubation time was 15 min. ○——○, 1-stearoyl-3-*sn*-GPC *plus* microsomal fraction; ○----○, 1-stearoyl-3-*sn*-GPC *plus* mitochondrial fraction; △——△, 2-oleoyl-3-*sn*-GPC *plus* microsomal fraction; △----△, 2-oleoyl-3-*sn*-GPC *plus* mitochondrial fraction.

TABLE I

CONVERSION OF 1-ACYL- AND 2-ACYL-3-*sn*-GPC INTO LECITHIN

About 300 nmoles of each 1-[9,10-$^3H_2$]stearoyl-3-*sn*-GPC and 2-[1-^{14}C]oleoyl-3-*sn*-GPC (Expt. A) or 2-[1-^{14}C]-linoleoyl-3-*sn*-GPC (Expt. B), 80 nmoles of the indicated fatty acids, 10 μmoles of $MgCl_2$, 20 μmoles of ATP, 0.4 μmole of CoA and 100 μmoles of Tris buffer (pH 7.2) were incubated for 15 min at 37° with 2 mg of rat liver microsomal protein in a final volume of 1.5 ml. Reactions were stopped by adding 5 ml of chloroform–methanol (1:2, v/v) and lipid extraction was completed according to BLIGH AND DYER[10]. Lecithin was isolated by thin-layer chromatography, converted into diglyceride by phospholipase C treatment and separated into the various molecular species by chromatography on silver nitrate-impregnated silica gel G plates according to methods described previously[11].

Expt.	*Fatty acids*	*Lysolecithins added*	*Molar ratios (1-acyl-3-sn-GPC/2-acyl-3-sn-GPC)*				*Distribution in lecithin (1-position/2-position)*	
			Lecithin	*Satd./polyunsatd. diglycerides*	*Satd./diunsatd. diglycerides*	*Satd./monounsatd. diglycerides*	[^3H]-*Stearate*	[^{14}C]*Oleate or* [^{14}C]-*linoleate*
A	Palmitate/linoleate	300/380 (0.79)	99/168 (0.59)	20.2/7.9 (2.56)	23.8/11.6 (2.05)	24.1/100.5 (0.24)	91/9	20/80
	Palmitate	300/380 (0.79)	83/158 (0.53)	21.9/6.5 (3.47)	13.4/9.6 (1.40)	21.0/95.4 (0.22)	91/9	16/84
	Linoleate	300/380 (0.79)	89/112 (0.80)	19.0/8.6 (2.20)	23.7/14.7 (1.61)	19.4/46.5 (0.42)	93/7	17/83
B	Palmitate/linoleate	360/300 (1.20)	190/230 (0.83)	55.2/17.2 (3.20)	54.5/127 (0.43)	13.0/16.3 (0.80)	96/4	9/91
	Palmitate	360/300 (1.20)	165/208 (0.79)	63.7/16.5 (3.86)	35.9/142.4 (0.25)	16.2/24.4 (0.66)	97/3	10/90
	Linoleate	360/300 (1.20)	171/175 (0.98)	48.5/20.9 (2.32)	45.2/87.5 (0.52)	11.6/14.6 (0.80)	97/3	20/80

a limited extent only (Table I). For example, when palmitate instead of a palmitate-linoleate mixture was used for the acylation of 1-[^{3}H]stearoyl-3-*sn*-GPC and 2-[^{14}C]-oleoyl-3-*sn*-GPC, a lowering of the molar ratios in the lecithin synthesized was indeed observed due to a preferential acylation of 2-[1-^{14}C]oleoyl-3-*sn*-GPC with palmitate.

However, a considerable amount of 1-[^{3}H]stearoyl-3-*sn*-GPC was still converted into lecithin under these conditions, apparently by acylation with endogenous unsaturated acyl chains from the microsomes. Acylation with endogenous fatty acids can be deduced also from the observation that the saturated/polyunsaturated species of the lecithin synthesized contained 20–30% of the [^{3}H]stearate irrespective of the fact that no polyunsaturated acids were added. These species also exhibited the highest molar ratios owing to the preferential acylation of the 1-[^{3}H]stearoyl-3-*sn*-GPC with polyunsaturated acids. As expected the lowest molar ratios were found in the molecular species formed by the acylation of the unsaturated 2-acyl-3-*sn*-GPC with a saturated acid. A considerable amount of the 1-[^{3}H]stearoyl-3-*sn*-GPC was also converted into these molecular species by acylation with either endogenous or added fatty acids. The isomeric lysolecithins are acylated without any appreciable migration of their acyl chains. The latter were recovered in the phosphatidylcholines at essentially the same positions at which they were esterified in the monoacyl phosphatidylcholines. The observed direct acylation of unsaturated 2-acyl-3-*sn*-GPC, preferentially with saturated fatty acids, supplements our knowledge of the important part these monoacyl phosphatidylcholines may play in the maintenance of the asymmetric distribution of saturated and unsaturated fatty acids during the renewal of acyl chains on species of phosphatidylcholine.

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Laboratory of Biochemistry,
The State University,
Utrecht (The Netherlands)

H. VAN DEN BOSCH
A. J. SLOTBOOM
L. L. M. VAN DEENEN

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