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Phospholipid Dependence of Rat Liver Microsomal Acyl : CoA Synthetase and Acyl-CoA : 1-Acyl-*sn*-Glycero-3-Phosphocholine O-Acyltransferase

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Summary. Investigations were performed on the influence of the phospholipid composition and physicochemical properties of the rat liver microsomal membranes on acyl-CoA synthetase and acyl-CoA : 1-acyl-*sn*-glycero-3-phosphocholine O-acyltransferase activities. The phospholipid composition of the membranes was modified by incubation with different phospholipids in the presence of lipid transfer proteins or by partial delipidation with exogenous phospholipase C and subsequent enrichment with phospholipids. The results indicated that the incorporation of phosphatidylglycerol, phosphatidylserine and phosphatidylethanolamine induced a marked activation of acyl-CoA synthetase for both substrates used—palmitic and oleic acids. Sphingomyelin occurred as specific inhibitor for this activity especially for palmitic acid. Palmitoyl-CoA: and oleoyl-CoA : 1acyl-*sn*-glycero-3-phosphocholine acyltransferase activities were found to depend on the physical state of the membrane lipids. The alterations in the membrane physical state were estimated using two different fluorescent probes—1,6-diphenyl-1,3,5-hexatriene and pyrene. In all cases of membrane fluidization this activity was elevated. On the contrary, in more rigid membranes obtained by incorporation of sphingomyelin and dipalmitoylphosphatidylcholine, acyltransferase activity was reduced for both palmitoyl-CoA and oleoyl-CoA. We suggest a certain similarity in the way of regulation of membrane-bound acyltransferase and phospholipase A_2 which both participate in the deacylation-reacylation cycle.

Key Words Acyl-CoA : LPC acyltransferase · acyl-CoA synthetase · microsomes · membrane fluidity · phospholipids

Introduction

It is well established that long-chain fatty acids have to be activated to acyl : CoA before being incorporated into glycerolipids. These processes are catalyzed by acyl : CoA synthetase (fatty acid : CoA ligase, EC 6.2.1.3) and acyl-CoA : 1-acyl-*sn*-glycero-3-phospholipid O-acyltransferase (EC 2.3.1.23).

Acyl-CoA synthetase has been reported in microsomes (Kornberg & Pricer, 1953), mitochondria (Norum, Farstad & Bremer, 1966; Garland, Yates & Haddock, 1970), peroxisomes (Shindo & Hashimoto, 1978) and plasma membranes (Davidson & Cantrill, 1986).

According to Pande and Mead (1968), there might be two different enzymes activating the saturated and unsaturated fatty acids. Other investigations, however, indicated that the activation of saturated and unsaturated fatty acids is carried out by one enzyme (Marcel & Suzue, 1972). It has also been established (Bar-Tana, Rose & Shapiro, 1971) that some exogenous factors could influence acyl : CoA synthetase activity. Singh and Poulos (1988) reported that detergent treatment of both microsomes and peroxisomes caused enzyme inhibition at a different degree. All phospholipids used by these authors—phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and sphingomyelin (SM)—also inhibited acyl : CoA synthetase.

Acyl-CoA : 1-acyl-*sn*-glycero-3-phosphocholine O-acyltransferase (acyl-CoA : LPC acyltransferase) (EC 2.3.1.23) is an important membrane-bound enzyme participating in the turnover of the phospholipid molecules and maintaining the membrane lipid composition (Stein, Widnell & Stein, 1968). Acyl-CoA : LPC acyltransferase activity has been reported in liver microsomes (Lands & Hart, 1965; Hill & Lands, 1970) and plasma membranes (Stahl & Trams, 1968; Colard et al., 1980) as well as in lung microsomes (Hasegawa-Sasaki & Ohno, 1975; Batenburg et al., 1979; Van Heusden, Vianen & Van den Bosch, 1980). This enzyme has been observed to display a definite substrate specificity in different animal tissues. For example, Lands et al. (1982) reported that liver microsomal acyltransferase shows a high specificity for unsaturated fatty acyl-CoA, whereas in lung microsomes, it prefers palmitoyl-CoA over oleoyl-CoA as a substrate (Sanford & Fronsolono, 1983).

Since acyl-CoA : LPC acyltransferase partici-

pates in modulation of the membrane phospholipid fatty acid composition, and thus, in maintenance of the membrane physical properties, it is of particular interest whether the membrane composition and physical state influence this activity. We have not come across in literature data devoted to this problem. Pugh and Kates (1984) reported that diets containing different saturated lipids did not alter the activity of microsomal acyltransferase. Investigations carried out in our laboratory (Momchilova-Pankova, Markovska & Koumanov, 1990) showed that the activity of acyl-CoA : LPE acyltransferase in rat liver plasma membrane depended on the membrane physical state.

In order to investigate in detail the effect of the membrane lipid environment on rat liver microsomal acyl : CoA synthetase and acyl-CoA : 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase activities, we modified the membrane's lipid composition and physicochemical properties. Using lipid transfer proteins (LTP) and exogenous phospholipase C, we managed to change both the phospholipid composition and the structural order of microsomal membranes, which made it possible to investigate the influence of these factors on the enzyme activities.

Materials and Methods

Animals

Male Wistar rats weighing about 200 g and fed standard laboratory diet were used in all experiments.

Reagents

We used phospholipase C (from *Clostridium perfringens*), 3-*sn*-phosphatidylethanolamine (PE), 3-*sn*-phosphatidylglycerol (PG), 3-*sn*-phosphatidylcholine from egg yolk (EYPC), N-acyl-4-sphingenyl-1-O-phosphorylcholine (SM), 3-*sn*-phosphatidylserine (PS), lyso-3-*sn*-phosphatidylcholine (LPC), palmitoyl-CoA and oleoyl-CoA (Sigma), 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine (DPPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphorylcholine (DOPC) (Fluka).

[1-^{14}C]oleoyl-CoA (50–60 mCi/mmol), [1-^{14}C]palmitoyl-CoA (50–60 mCi/mmol), [1-^{14}C]palmitic acid (50–60 mCi/mmol) and [1-^{14}C]oleic acid (50–60 mCi/mmol) were purchased from Amersham (UK).

Isolation of Rat Liver Microsomes

Rat liver microsomes were isolated by differential centrifugation at 105,000 × *g* in 0.1 M Tris-HCl, pH 7.4.

Partial Purification of LTP

The postmitochondrial 105,000 × *g* supernatant was adjusted to pH 5.1 with 3 N HCl. After 1 hr, the precipitate was sedimented by centrifugation for 15 min at 14,000 × *g* in a Janetzki K-24 refrigerated centrifuge (GDR) and discarded. The pH of the supernatant was readjusted to 7.4 with 1 M Tris-HCl. Solid ammonium sulfate was added slowly to the supernatant to 90% saturation, and the mixture was stirred overnight. The precipitate was sedimented by centrifugation for 15 min at 14,000 × *g*, dissolved in 5 mM sodium phosphate/10 mM β-mercaptoethanol (pH 7.2) and dialyzed for 24 hr against the same buffer. Protein precipitated during dialysis was removed by centrifugation. The supernatant was used as LTP source.

Enrichment of Microsomes with Phospholipids

Incubations were performed for 1 or 2 hr at 37°C in the presence of partially purified lipid transfer proteins (LTP). The incubation medium contained LTP/membrane protein/phospholipid liposomes at a ratio 1 : 1 : 0.4 (by weight) in a 0.25 M sucrose (0.01 M) EDTA (0.1 M) Tris-HCl buffer, pH 7.4. LTP of different origins were used for enrichment of the membrane fractions as follows: (i) for enrichment with sphingomyelin (SM)—LTP from chicken liver; (ii) for enrichment with DPPC, EYPC and PG—LTP from rat lung; and (iii) for enrichment with DOPC, PE, PS and PI—LTP from rat liver.

The enriched membranes were washed twice with 10 mM Tris-HCl buffer, pH 7.4 and used for further investigations.

Electron microscopy studies showed that there were no liposomes attached to the membrane fractions after double washing.

Treatment of Liver Microsomes with Exogenous Phospholipases C and Subsequent Enrichment with Phospholipids

Incubations were performed with exogenous phospholipase C for 20 min at 37°C as follows: 5 units of the enzyme were incubated with microsomes (1 μM phospholipids) in 5 mM $CaCl_2$, 10 mM Tris-HCl buffer, pH 7.4. The reaction was terminated by chilling and centrifugation of microsomes in a Sorvall OTD-50 ultracentrifuge at 105,000 × *g* for 60 min at 4°C. The pellet was washed twice in the same buffer, and the partially delipidated membranes were used for enrichment with different phospholipids.

Acyl : CoA Synthetase Assay

The standard reaction mixture contained: 0.1 M Tris-HCl (pH 8.0), 1.6 mM Triton X-100, 5 mM dithiothreitol, 150 mM KCl, 15 mM $MgCl_2$, 10 mM ATP, 16 nmol [1-^{14}C] palmitic (10,000 cpm/sample) or [1-^{14}C] oleic acid (8,000 cpm/sample), 0.6 mM CoA and 50 μg membrane protein in a total volume 0.2 ml. The reaction was initiated by addition of membranes and was carried out for 5 min at 37°C. Termination was performed by addition of 2.25 ml of isopropanol-hexane : 1 M $H_2SO_4^-$ (40 : 10 : 1 by vol). Hexane (1.5 ml) and 1 ml H_2O were added to each sample, and the upper

phase was removed. The lower phase was washed twice to remove the unesterified fatty acids. The radioactivity of the water-soluble product-labeled acyl : CoA was counted on a LKB Rack-Beta II-1215 scintillation counter. The specific activity of the enzyme was expressed as nmol of fatty acyl : CoA formed per min per mg protein.

Acyltransferase Assay (Iritani, Ikeda & Kajitani, 1984)

The standard reaction mixture (0.1 ml) contained: 100 mM Tris-HCl (pH 7.5), 100 μmol acyl-CoA thioester (8,000 cpm/sample), 0.5% (wt/vol) albumin and 50 μg microsomal protein. Lysophosphatidylcholine (100 μmol) was preincubated with the membrane fraction for 15 min at 30°C. Incubations were performed for 5 min at 37°C. The reaction was started by addition of the acyl-CoA thioesters and was stopped by addition of 2 ml $CHCl_3/CH_3OH$ (2 : 1, vol/vol). The chloroform phase was dried in scintillation vials and counted on Rackbeta II-1215 (LKB) scintillation counter. Control incubations were always conducted in the absence of acyl-acceptor, and the observed rates were subtracted from the rates measured in the presence of acyl-acceptor which eliminates the eventual interference of acyl-CoA hydrolase activity.

Acyltransferase activity was expressed as nmol of transferred fatty acid per min/mg protein.

Analytical Methods

Lipids were extracted from microsomes by the method of Folch, Lees and Sloane-Stanley, 1975). The phospholipid content was determined in the total lipid extract (Kahovkova & Odavic, 1969). The individual phospholipids were chromatographed on silica gel 60 thin-layer plates (Merck). Chloroform/methanol/isopropanol/0.25% KCl/triethylamine (30 : 9 : 25: 6 : 18, by vol) were used as developing solvents (Touchstone, Chen & Beaver, 1980). The membrane protein content was determined according to Lowry et al. (1951).

Fluorescence Assays

Fluorescence studies were performed on freshly obtained microsomes, either by fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH) or pyrene excimer formation.

DPH (Fluka) was used as a fluorescent probe for estimation of microsomal structural order. The steady-state anisotropy of fluorescence (rs) of DPH was estimated from:

$$rs = I_{II} - I_{-}/I_{II} + 2I_{\perp}$$

(Shinitsky & Barenholz, 1974).

The lipid structural order parameter (S_{DPH}) was calculated by an empirical method described by Van Blitterswijk, Van Hoeven and Van der Meer (1981).

Fluorescent measurements have been performed at 355 (excitation beam) and 425 nm (emission beam).

The other fluorescent method is based on the finding that the formation of excited pyrene dimers (excimers) in fluid membranes is a diffusion-controlled process (Galla & Sackmann, 1974; Dix & Verkman, 1990).

Excitation wavelength was 320 nm, and emission wavelengths were 380 (monomer) and 465 nm (excimer). The pyrene-to-lipid molar ratio was 0.0147.

Fluorescent measurements were performed on a Perkin-Elmer 3000 fluorescence spectrometer.

Statistical analysis was performed using Student's t test.

Results

The incubation of microsomes with different phospholipids in the presence of LTP induced significant alterations in the phospholipid composition (Fig. 1). We observed an augmentation of the level of all phospholipid fractions.

On the background of the alterations in the phospholipid composition we investigated the activity of acyl-CoA synthetase and acyl-CoA : LPC acyltransferase. The results presented in Table 1 indicate that the activity of acyl-CoA synthetase was higher when oleic acid was used as substrate, in comparison with palmitic acid. The enrichment of microsomal membranes with SM caused a strong inhibition of this activity for both substrates. The inhibition of palmitoyl : CoA synthetase activity was most pronounced, reaching almost 90%. All three molecular species of PC—DPPC, DOPC and EYPC—did not influence significantly acyl : CoA synthetase activity. However, the incorporation of PE, PS and PG induced acyl : CoA synthetase activation. PG displayed a most marked activating effect.

The changes in acyltransferase activity in modified membranes, using two different substrates—palmitoyl-CoA and oleoyl-CoA—are shown in Fig. 2. In control membranes this enzyme activity was about twice as high towards the unsaturated oleoyl-CoA. However, the alterations in the enzyme-specific activity for both substrates were quite similar. The enrichment of microsomal membranes with SM and DPPG caused a reduction of acyltransferase activity, whereas enrichment with PS, DOPC, PE, EYPC and PG induced the opposite effect.

We observed, that the alterations of acyltransferase activity correlated well with the changes of the membranes physical state, caused by the incorporated phospholipids. For example, SM and DPPC, which were found to inhibit acyltransferase activity, induced a reduction of microsomal membranes fluidity (illustrated by the decrease of the I_e/I_m ratio and the increase of S_{DPH}), whereas the other phospholipids, which activated the enzyme, caused a different degree of membrane fluidization (increase of the I_eI_m ratio and decrease of S_{DPH}) (Fig. 2). The correlation coefficients between acyltransferase activity and the I_e/I_m ratio were 0.951 and 0.953 for

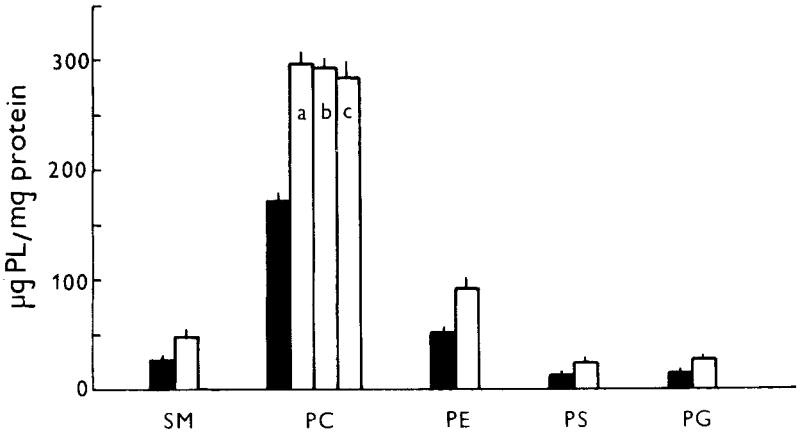

Fig. 1. Phospholipid level (μg PL/mg protein) in native (control) (filled bar) and enriched with phospholipids by the aid of LTP (open bar) microsomal membranes. Values are means ± SD of duplicates in two separate experiments. *SM*, sphingomyelin; *PC*, phosphatidylcholine; *PE*, phosphatidylethanolamine; *PS*, phosphatidylserine; *PG*, phosphatidylglycerol; and *PL*, phospholipids. *a*, enrichment with dipalmitoyl-PC; *b*, enrichment with dioleoyl-PC; and *c*, enrichment with egg yolk PC.

Table 1. Specific activity of acyl-CoA synthetase in microsomal membranes enriched with different phospholipids by the aid of lipid transfer proteins (nmol · min^{-1} · mg^{-1})[a]

Enrichment	$C_{16:0}$	$C_{18:1}$
Control	58.44 ± 2.18	87.47 ± 2.94
SM	7.06 ± 1.33[c]	46.29 ± 2.87[c]
DPPC	53.41 ± 2.03[c]	81.11 ± 3.08[b]
EYPC	55.99 ± 1.87	85.09 ± 2.09
DOPC	57.26 ± 2.08	81.36 ± 3.08
PE	85.01 ± 2.12[c]	98.19 ± 2.15[c]
PS	73.50 ± 1.15[c]	134.28 ± 4.67[c]
PG	131.19 ± 3.68[c]	160.86 ± 4.63[c]

[a] Detailed experimental conditions and procedures are described under Materials and Methods.
Values are means ± SD of duplicates in two separate experiments.
[b] $P < 0.05$, [c] $P < 0.001$.
SM, sphingomyelin; DPPC, dipalmitoylphosphatidylcholine; EYPC, egg yolk phosphatidylcholine; DOPC, dioleoylphosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PG, phosphatidylglycerol.

palmitoyl-CoA and oleoyl-CoA, respectively. Between acyltransferase activity and S_{DPH}, these coefficients were −0.950 and −0.935.

Partial delipidation of microsomal membranes, using exogenous phospholipase C and subsequent enrichment with different phospholipids, yielded series of membranes with various phospholipid composition (Fig. 3), which allowed us to determine the influence of definite phospholipids on the enzyme activity.

Pretreatment of the membranes with exogenous phospholipase C caused an insignificant reduction of synthetase activity for both fatty acids (Table 2). The enrichment with SM leads to a further inhibition of this activity. However, the incorporation of different molecular species of PC did not alter synthetase activity. PE, PS and especially PG, restored at a different degree this activity which has been reduced due to phospholipase C treatment.

Acyltransferase activity was reduced due to phospholipase C treatment by about 50% for both substrates used (Fig. 4). Enrichment of such membranes with SM and DPPC induced a further decrease of this activity, whereas DOPC, EYPC, PE and the other phospholipids used induced enzyme activation which was most pronounced in membranes enriched with PS (about twice the activity in phospholipase C-treated microsomal membranes). Such augmentation was observed for both substrates, palmitoyl-CoA and oleoyl-CoA (Fig. 4).

It is evident from Fig. 4 that treatment of microsomes with exogenous phospholipase C caused a reduction of membrane fluidity. The incorporation of DOPC, EYPC, PS, PE and PG into partially delipidated microsomal membranes induced a marked fluidization, whereas the incorporation of SM and DPPC could not cause any further decrease of membrane fluidity. In this case we also observed correlation between acyltransferase activity and the I_e/I_m ratio (r = 0.883 and 0.897 for palmitoyl-CoA and oleyl-CoA, respectively). Between acyltransferase and S_{DPH} the correlation coefficients were −0.839 and −0.973.

Discussion

The formation of fatty acyl-CoA is a potential regulatory step in the process of phospholipid reacylation (Miyazawa, Hashimoto & Yokota, 1985). Acyl-CoA is usually formed by acyl-CoA synthetase using as substrate free fatty acids either synthesized *de-novo* or derived from dietary sources.

As already mentioned, this enzyme activity is known to be influenced by some exogenous factors as well as by the presence of definite phospholipids.

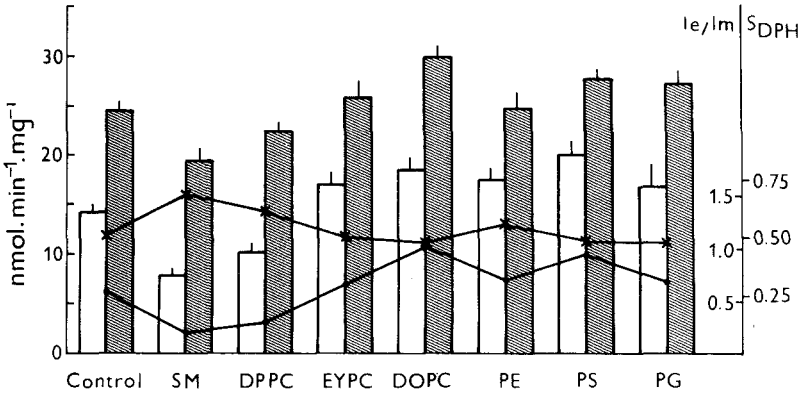

Fig. 2. Palmitoyl-CoA: (open bar) and oleoyl-CoA : LPC acyltransferase activity (striped bar), S_{DPH} (*), and the I_e/I_m ratio (●) in microsomeal membranes enriched with different phospholipids by the aid of LTP. Values are means ± SD of duplicates in two separate experiments. All observed alterations in S_{DPH} and the I_e/I_m ratio after enrichment of microsomal membranes with phospholipids were statistically significant. Abbreviations are as in Fig. 1.

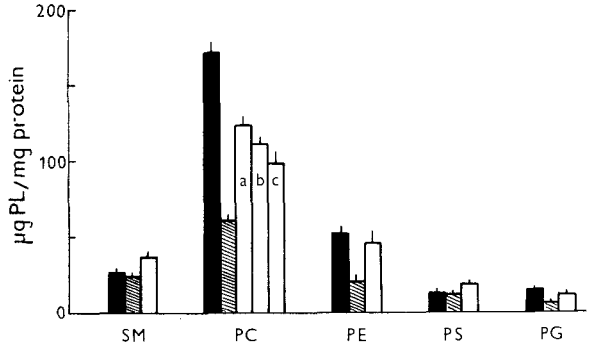

Fig. 3. Phospholipid level (µg PL/mg protein) in the native (control) (filled bar), phospholipase C-treated (striped bar) and enriched with phospholipids after phospholipase C treatment (open bar) microsomal membranes. Values are means ± SD of duplicates of two separate experiments. Abbreviations are as in Fig. 1.

Table 2. Specific acitivity of acyl-CoA synthetase in microsomal membranes partially delipidated with exogenous phospholipase C (PLase C) and subsequently enriched with different phospholipids (nmol · min^{-1} · mg^{-1})[a]

Enrichment	$C_{16:0}$	$C_{18:1}$
PLase C-treated	51.38 ± 2.31	83.05 ± 2.52
SM	32.14 ± 3.05[c]	78.54 ± 1.85
DPPC	51.45 ± 3.19	84.42 ± 1.89
EYPC	52.73 ± 2.77	84.16 ± 3.07
DOPC	54.16 ± 1.09	83.77 ± 2.17
PE	57.84 ± 3.02[b]	87.45 ± 1.57[b]
PS	57.31 ± 1.81[b]	89.12 ± 1.98[c]
PG	64.31 ± 3.31[c]	93.67 ± 2.63[c]

[a] Detailed experimental conditions and procedures are described under Materials and Methods.
Values are means ± SD of duplicates in two separate experiments.
[b] $P < 0.01$; [c] $P < 0.001$.
PLase C-treated membranes were used as controls for estimation of the statistical significance.

Singh and Poulos (1988) have studied the influence of various phospholipids on the activity of acyl-CoA synthetase. They reported that PC, PE, PI and SM inhibited the enzyme when added to the incubation medium. It should be noted that the difference between our approach for investigation of the factors affecting acyl-CoA synthetase activity and the one used by Singh and Poulos (1988) is that we studied the influence of definite phospholipids located in the enzyme lipid surrounding, and not added directly to, the incubation medium.

As evident from our results, SM was the only membrane lipid which exhibited a marked inhibitory effect on both palmitoyl-CoA and oleoyl-CoA synthetase activities in rat liver microsomes. However, the inhibitory effect of SM was not related to changes in membrane fluidity, because the other phospholipid which decreased membrane fluidity, DPPC, did not affect acyl-CoA synthetase activity. Treatment of membranes with exogenous phospholipase C, which also increased membrane rigidity, had almost no effect on this activity. Most probably the effect of SM could be due to the specific structure of this sphingophospholipid, which differs markedly from the structure of the other glycerophospholipids building up the lipid bilayer. Moreover, membrane enrichment with the three different molecular species of PC, DPPC, DOPC and EYPC, which alter the membrane structural order in different directions, did not influence significantly the two investigated acyl-CoA synthetase activities. However, we observed a definite dependence on some other phospholipid fractions, such as PG, PS and PE. PG appeared to be the most effective activator.

The selective incorporation of fatty acids after being activated to acyl-CoA into phospholipids is known to be carried out by specific acyl-CoA : lysophospholipid acyltransferase (Okuyama & Lands, 1972; Iritani et al., 1984).

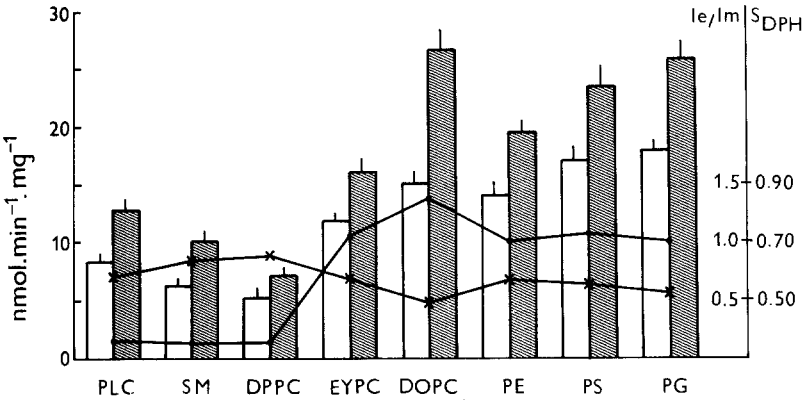

Fig. 4. Palmitoyl-CoA: (open bar) and oleoyl-CoA : LPC acyltransferase activity (striped bar), S_{DPH} (*), and the I_e/I_m ratio (●) in microsomeal membranes partially delipidated with exogenous phospholipase C (PLC) and subsequently enriched with different phospholipids. Values are means ± SD of duplicates in two separate experiments. Statistically significant alterations were observed as follows: (i) for S_{DPH}, (ii) for enrichment with all phospholipids, (iii) for the I_e/I_m ratio, and (iv) for enrichment with EYPC, DOPC, PE, PS and PG. Abbreviations are as in Fig. 1.

In our previous paper (Momchilova-Pankova et al., 1990), we reported that liver plasma membrane acyl-CoA : LPE acyltransferase is sensitive towards changes in membrane fluidity. Membrane fluidization due to enrichment of plasma membranes with DOPC, EYPC, PE, PS and PG induced enzyme activation. On the contrary, decrease of fluidity caused by incorporation of SM and DPPC was accompanied by reduction of this activity.

As evident from the results presented in this paper, acyl-CoA : LPC acyltransferase in microsomal membranes displayed a similar behavior. The incorporation of phospholipids inducing elevation of the membrane structural order, such as SM and DPPC, caused a significant reduction of this activity. Partial delipidation of microsomal membranes by the aid of exogenous phospholipase C, which also resulted in decrease of membrane fluidity, exhibited a similar effect on acyltransferase. This effect of phospholipase C treatment was due to the removal of the polar head groups of some of the membrane phospholipids, as well as to reduction of the membrane surface charge. In addition, the accumulation of diacylglycerols, which have molecules of a specific shape, facilitates the formation of more rigid lipid layers. We reported a similar effect of phospholipase C treatment in previous papers (Momchilova, Petkova & Koumanov, 1986; Momchilova-Pankova et al., 1990).

The incorporation into the membranes (native or partially delipidated) of phospholipids inducing membrane fluidization, on the contrary, caused activation of acyltransferase. Such effect was displayed by DOPC, EYPC, PE, PS and PG.

The correlation between membrane fluidity and acyltransferase activity was most pronounced in membranes enriched with the three different molecular species of PC, DPPC, EYPC and DOPC, which alter membrane fluidity in opposite directions due to their specific fatty acid composition. As evident from Figs. 2 and 4, the elevation of membrane fluidity from DPPC to EYPC and DOPC induced a gradual activation of the acyl transfer.

These data support the idea that membrane fluidity is essential for acyltransferase activity and not the presence of a definite phospholipid fraction. It is noteworthy that the effect of the membrane physical state was observed for both of the substrates used, palmitoyl-CoA and oleoyl-CoA.

It is not unlikely that the dependence of acyltransferase activity on the membrane physical state is related to the influence of membrane fluidity on membrane-bound phospholipase A_2 reported in a previous paper (Petkova, Momchilova-Pankova & Koumanov, 1987). The activation of phospholipase A_2 due to membrane fluidization induces elevation of the level of lysoPC, which participates in the acyltransferase-catalyzed reaction as an acceptor of the fatty acids. Colard et al. (1980) reported that the elevation of the 1-acyl-lysophosphatidylcholine levels induced augmentation of linoleate and oleate incorporation into the membrane phospholipids. However, certain similarities in the way of regulation of the two enzymes participating in the deacylation-reacylation cycle—phospholipase A_2 and acyltransferase—seems quite logical.

On the other hand, the eventual role of the augmented lateral diffusion of the substrate and enzyme molecules in more fluid membranes which increases the number of contacts between them, should not be ruled out. As evident from this paper, the augmented acyltransferase activity was always accompanied by a corresponding elevation of the I_e/I_m ratio, which can be used for estimation of the lateral diffusion (Galla & Hartman, 1980). Most probably the regulation of the deacylation-reacylation cycle is quite complex and is subjected to various biochemical and biophysical factors, but apparently the effect of the physical state of the membrane lipid bilayer should be taken into consideration.

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