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LYSOLECITHIN AS REGULATOR OF *DE NOVO* LECITHIN SYNTHESIS IN RAT LIVER MICROSOMES

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SUMMARY

1-Acyl-*sn*-glycero-3-phosphocholine (lysolecithin) was found to affect 1,2-diacyl-*sn*-glycerol:CDPcholine cholinephosphotransferase (CPT; EC 2.7.8.2) activity of rat liver microsomes in a concentration dependent, characteristic manner. Cholinephosphate transfer was activated at lysolecithin concentrations below 0.5 mM with a maximum stimulation occurring at 75-100 μM lysolecithin levels. At concentrations above 0.5 mM, CPT activity was inhibited by lysolecithin. It was shown that CPT inhibition by lysolecithin is competitive (Ki ≈ 0.6 mM) with respect to CDPcholine. The possible role of lysolecithin as regulator of *de novo* lecithin synthesis *in vivo* is outlined.

The final step in the *de novo* synthesis of lecithin is catalyzed by 1,2-diacyl-*sn*-glycerol:CDPcholine cholinephosphotransferase (CPT; EC 2.7.8.2) (1). This enzyme is membrane-bound and was shown to be located on the cytoplasmic side (2) of the endoplasmic reticulum (3). CPT has been solubilized from rat liver microsomes and purified several-fold (4). *In vitro* CPT activity is known to be inhibited by calcium, Triton X-100, Triton WR 1339, Tween 20, CDP, and CDPethanolamine (5), as well as by palmitoyl-CoA (5,6), centrophenoxine (7), or by phospholipase A_2 treatment (8). On the other hand, sodium deoxycholate was found to stimulate cholinephosphotransferase and was utilized in CPT purification (4).

We now report on our observation that rat liver microsomal cholinephosphotransferase activity is stimulated by low levels of lysolecithin and inhibited by higher lysolecithin concentrations. Implications of these findings and the possible regulatory function of lysolecithin in lecithin synthesis are discussed.

Abbreviations: CDP, cytidine 5'-diphosphate; CPT, 1,2-diacyl-*sn*-glycerol:CDPcholine cholinephosphotransferase [EC 2.7.8.2]; lysoPC; 1-acyl-*sn*-glycero-3-phosphocholine, lysolecithin; TLC, thin-layer chromatography; EGTA, ethyleneglycol-*bis*(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid.

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MATERIALS AND METHODS

1,2-Diacyl-*sn*-glycerol was prepared by hydrolysis of rat liver phosphatidylcholine with phospholipase C from *Chlostridium welchii* (9) in a modified incubation system (10). The phosphatidylcholine used was isolated from the total lipids of rat liver (11) by preparative thin-layer chromatography; developing solvent, chloroform-methanol-water, 65:25:4 (by vol). 1-Hexadecanoyl-*sn*-glycero-3-phosphocholine was prepared from 1,2-dihexadecanoyl-*rac*-glycero-3-phosphocholine (United States Biochemical Corp., Cleveland OH) by action of phospholipase A_2 (*Ophiophagus hannah* venom, Miami Serpentarium Laboratories, Miami, FL) according to established procedures (12). The 1-hexadecanoyl-*sn*-glycero-3-phosphocholine was isolated by preparative thin-layer chromatography from the partially hydrolyzed lipid mixture using chloroform-methanol-water, 65:35:8 (by vol) as developing solvent. The purified lysolecithin migrated as uniform fraction in TLC (R_f 0.2) and showed an optical rotation of $[\alpha]^{25}_{546.1}$-2.95° (c, 8.8 in chloroform-methanol, 9:1, v/v); literature values $[\alpha]^{25}_{D}$-2.87° (c, 10; ref 13), $[\alpha]^{25}_{D}$-2.9° (ref 14). The 1-acyl-*sn*-glycero-3-phosphocholine structure was verified by carbon-13 nuclear magnetic resonance spectroscopy on a Varian FT-80A Fourier transform instrument operating at 20 MHz; solvent, $CDCl_3$:CD_3OD:D_2O, 50:50:15 (by vol); chemical shifts of noise-decoupled resonances are given downfield (δ, ppm) from Me_4Si: 54.44 (t, Me_3N, J_{NC} 3.4 Hz), 59.68 (d, P-O-CH_2 of choline, J_{POC} 5.1 Hz), 65.52 (s, *sn*-1 CH_2-O-CO-R), 66.83 (m, CH_2N), 67.20 (d, *sn*-3 CH_2-O-P, J_{POC} 5.6 Hz), 69.06 (d, *sn*-2 CH-OH, J_{POCC} 7.3 Hz), 175.16 (s, C=O), and customary aliphatic signals. A *sn*-2 carbon signal for CH-O-CO-R (lecithin, 71.0 ppm) was absent (Murari, R., Wedmid, Y. and Baumann, W. J., unpublished data).

Liver microsomes were prepared from male Sprague-Dawley rats (200 g) according to Fleischer and Kervina (15) as described previously (7). Protein was determined by the method of Lowry *et al.* (16) using crystalline bovine serum albumin as standard.

1,2-Diacyl-*sn*-glycerol:CDPcholine cholinephosphotransferase [EC 2.7.8.2] activity was determined according to Coleman and Bell (5). The incubation system (final volume, 200 µl) contained 30 µg of rat liver microsomal protein in 175 mM Tris/Cl buffer (pH 8.5), 8 mM $MgCl_2$, 100 µM CDP-[*methyl*-^{14}C]choline (2.5 µCi/µmol; 5500 dpm/nmol; Amersham Corp., Arlington Heights, IL; unlabeled carrier was from Sigma Chemical Co., St. Louis, MO), 500 µM EGTA, and 100 nmol of rat liver 1,2-diacyl-*sn*-glycerol dissolved in 5 µl of absolute ethanol (final concentration, 500 µM). Bovine serum albumin was omitted. After incubating for 10 min at 37° C, the reaction was terminated by addition of 0.6 ml of 1% $HClO_4$, phospholipids were extracted by the method of Bligh and Dyer (11) using 3 ml of chloroform-methanol, 1:2 (v/v), followed by 1 ml of chloroform and 1 ml of 1% $HClO_4$. The lower phase was washed three times with 1 ml of 1% $HClO_4$ and an aliquot of the chloroform phase was transferred to a scintillation vial, dried, and radioactivity was determined using 10 ml of scintillation fluid prepared from 12 g of omnifluor (New England Nuclear Corp., Boston, MA), 1000 ml of Triton X-100 and 2000 ml of toluene. Results given are averages of at least two independent assays.

RESULTS

1,2-Diacyl-*sn*-glycerol:CDPcholine cholinephosphotransferase activity of rat liver microsomes was measured by following the incorporation of label from CDP-[*methyl*-^{14}C]choline into lecithin using 1,2-diacyl-*sn*-glycerol as acceptor. In the absence of exogenous lysolecithin, the incubation system produced about 230 nmol of lecithin per hr per mg microsomal protein. Figure 1 illustrates

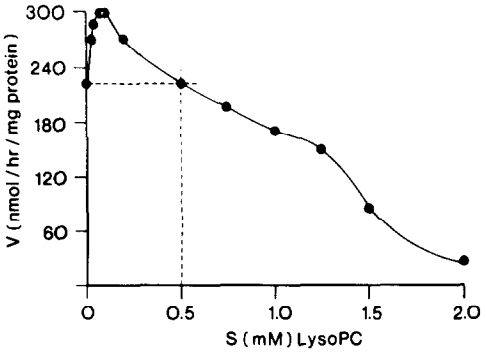
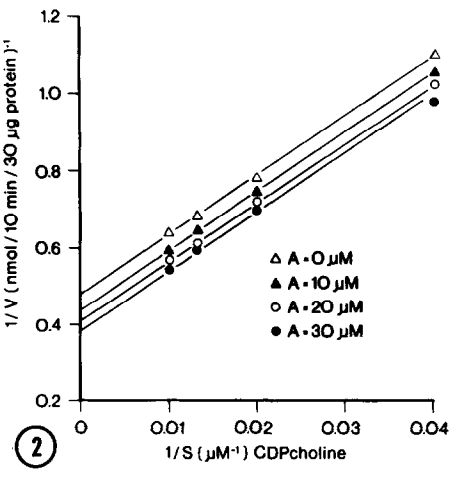


Figure 1. Effect of lysolecithin on CPT activity of rat liver microsomes. Incubations were carried out using 30 µg of microsomal protein as described in Materials and Methods. Lysolecithin was added as a 0.01 M solution in Tris/Cl buffer.

the effect of lysolecithin supplementation to the system. It is apparent that addition of 1-hexadecanoyl-*sn*-glycero-3-phosphocholine at concentrations lower than 0.5 mM consistently resulted in CPT activation with a maximum stimulation (≈ 300 nmol/hr/mg) occurring at 75-100 µM lysolecithin levels. However, when CPT activity was followed as function of lysolecithin concentrations above 0.5 mM, cholinephosphate transfer to diglyceride became progressively inhibited, and inhibition was essentially complete at 2 mM lysolecithin (see Figure 1). Excess microsomal protein up to 100 µg did not affect percentage inhibition.

The kinetics of CPT activation and of CPT inhibition at representative lysolecithin concentrations with CDPcholine as substrate were followed and the results are given in Figures 2 and 3. The double reciprocal plot (1/V versus 1/S) of the activation phase (Figure 2) gave a Km of 45 µM for CDPcholine in the absence of exogenous lysolecithin; a Km of about 85 µM was observed at 30 µM lysolecithin corresponding to a stimulation of CPT activity by about 20%. The double reciprocal plot of the inhibition phase (Figure 3) showed a Km for CDPcholine that increased from 45 µM with increasing concentrations of lysolecithin in the medium. Vmax, on the other hand, remained constant. From Figure 3 it is inferred that at the concentrations given, lysolecithin inhibits the cholinephosphotransferase reaction *competitively* with respect to CDPcholine. A Ki of about 0.6 mM was determined from a replot of the slopes versus inhibitory lysolecithin concentrations.



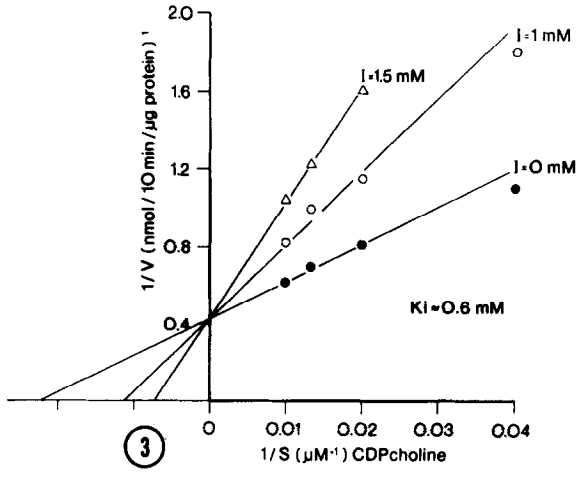


Figure 2. Double reciprocal plot of CPT activation by lysolecithin. Various concentrations of lysolecithin (activator A) were added as 0.01 M solutions in Tris/Cl buffer.

Figure 3. Double reciprocal plot of CPT inhibition by lysolecithin. Various concentrations of lysolecithin (inhibitor I) were added as 0.01 M solutions in Tris/Cl buffer. The Ki given (0.6 mM) was determined from the replot of the slopes against inhibitor concentrations.

DISCUSSION

The data demonstrate that lysolecithin can affect CPT-catalyzed *de novo* lecithin synthesis by rat liver microsomes either by activating or inhibiting the reaction depending on the lysolecithin concentration. It is tempting, of course, to attribute the effect of lysolecithin to its detergent properties. Other detergents, such as Triton X-100 and WR 1339, Tween 20, or palmitoyl-CoA, have previously been shown to exert an inhibitory effect on CPT (5,6); on the other hand, deoxycholate was shown to activate CPT (4). However, our data show that inhibition of CPT by lysolecithin above 0.5 mM concentrations is competitive in nature. Competitive inhibition in respect to CDPcholine cannot be explained by detergent properties *per se*. Hence, alternate modes of inhibition should be considered, including rather specific lysolecithin-enzyme interactions. In contrast, activation of CPT at micromolar lysolecithin concentrations may involve less specific interactions of lysolecithin with the microsomal structure probably resulting in more subtle changes on the protein.

Modulation of CPT by lysolecithin is also noteworthy in view of the close metabolic interrelationship between lysolecithin and lecithin, particularly

through the lecithin deacylation-reacylation cycle (17). Thus, inhibition of CPT at high lysolecithin levels may well serve the purpose of shutting off *de novo* lecithin synthesis from diglyceride in order to channel excess lysolecithin into lecithin synthesis via lysolecithin acyltransferases. Alternatively, low lysolecithin levels would switch on CPT and *de novo* lecithin synthesis in order to maintain lecithin production without depletion of the lysolecithin pool.

Whether lecithin metabolism in liver is actually regulated by lysolecithin remains to be tested, of course, although evidence has begun to emerge from studies carried out on intestine that would support this concept. Thus, Mansbach and Parthasarathy (18) have shown that upon intestinal infusion of lecithin, which is absorbed as lysolecithin (19), ^{32}P incorporation into lecithin decreased. While this was partially attributed to changes in CDPcholine synthesis (18,20), it was clearly shown *in vitro* that the kinetics of the CPT reaction were affected upon lecithin infusion (18).

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