Lysophosphatidylcholine Metabolism in the Rabbit Heart

CHARACTERIZATION OF METABOLIC PATHWAYS AND PARTIAL PURIFICATION OF MYOCARDIAL LYSOPHOSPHOLIPASE-TRANSACYCLASE*

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Metabolism of lysophosphatidylcholine (LPC), recently implicated in arrhythmogenesis, was characterized in rabbit ventricular homogenates. Activities of four enzymatic pathways were distinguishable after subcellular fractionation and DEAE-Sephacel chromatography including microsomal lysophospholipase, microsomal acyl coenzyme A/LPC acyltransferase, cytosolic lysophospholipase, and cytosolic lysophosphatidylcholine synthase. Microsomal lysophospholipase activity was attenuated 81% by acidosis comparable to that in ischemic myocardium (pH 6.5) and was inhibited by substrate. LPC acyltransferase was identified in the microsomal fraction based on CoA-dependent phosphatidyl choline synthesis, the positional specificity of acylation of LPC, and identical reaction velocities with both of its labeled co-substrates. LPC acyltransferase had a Vmax of 5.1 nmol/mg/min, a broad pH optimum centered at pH 7, and an apparent Km for LPC and palmitoyl-CoA of 14 μM and 7 μM. Cytosolic lysophospholipase was separated from lysophospholipase-transacylase by DEAE-Sephacel chromatography and distinguished from microsomal lysophospholipase by its broad pH activity curve, Michaelis-Menten kinetics (Vmax = 9.5 nmol/μg/min, Km = 7.5 μM), and lack of substrate inhibition. Lysophospholipase-transacylase was identified in the cytosolic fraction by CoA-independent phosphatidyl choline synthesis and purified 4885-fold from homogenate by ammonium sulfate precipitation, DEAE-Sephacel, hydroxylapatite, gel filtration, and polylysine chromatography. The partially purified enzyme had a transacylase/lysophospholipase activity ratio of 0.6, and transacylation of LPC was prominent at submicellar concentrations of substrate.

Amphiphiles, including long chain acylcarnitine and lysophosphatidylcholine, have been implicated as biochemical mediators of electrophysiological derangements in ischemic myocardium potentially contributing to malignant ventricular dysrhythmias (1, 2). Such compounds alter electrophysiological behavior of canine Purkinje fibers and of ventricular muscle in a fashion closely analogous to that of membrane phospholipid when as little as 2% of membrane phospholipid is constituted with amphiphiles acquired from exogenous sources (3). Thus, accumulation of even small amounts of LPC may alter the biophysical characteristics of cardiac membranes and give rise to electrophysiological perturbations similar to those seen in ischemic myocardium.

Early after the onset of myocardial ischemia phospholipid metabolism appears to be altered. Lysophosphatidylcholine accumulates in ischemic zones (4, 5) and in venular effluents from ischemic regions (6). Such accumulation may reflect: 1) increased production, 2) decreased metabolism, 3) diminished washout, or 4) combinations of these phenomena. Although rabbit myocardial phospholipid activity is only modest (7), the overall capacity for metabolic clearance of LPC in rabbit myocardium was found to be large in preliminary experiments in the present study. Thus, the amount of LPC potentially capable of interacting with membranes may vary considerably depending on relatively modest changes in the overall rates of metabolic clearance.

Although metabolism of lysophospholipids has been studied extensively in liver (e.g. Refs. 8–10), lung (e.g. Refs. 11 and 12), and brain (e.g. Refs. 13 and 14), little information is available characterizing LPC metabolism in myocardium. It is likely that phospholipid metabolism differs markedly in the heart compared to the liver in view of differences of profiles of products accumulating in the two organs when they are rendered ischemic (4, 15). Accordingly, the present study was undertaken to identify metabolic pathways of LPC in rabbit heart and determine kinetic characteristics of competing and interacting pathways to gain insight into specific metabolic mechanisms potentially responsible for accumulation of lysophosphatidylcholine in ischemic myocardium. Four enzymatic pathways of rabbit myocardial LPC metabolism were identified and characterized including: 1) acyl coenzyme A/LPC acyltransferase (EC 2.3.1.23) (Fig. 1, top left) present in the microsomal fraction of rabbit ventricular homogenates, exhibiting specific substrate requirements, distinctive positional specificity of acylation, and identical reaction velocities when either of its co-substrates was labeled; 2) lysophospholipase (EC 3.1.1.5) (Fig. 1, bottom left) in the microsomal fraction, characterized by kinetic parameters, pH profile, and substrate inhibition; 3) lysophospholipase in the cytosolic fraction separated from lysophospholipase-transacylase activity by ion exchange chromatography and characterized by its pH profile, maximum velocity, and substrate dependence; and 4) lysophospholipase-transacylase (EC 3.1.1.5) (horizontal reaction Fig. 1) identified in the cytosolic fraction based on CoA-independent phosphatidylcholine synthesis, co-purification of FA and PC synthetic activities, and characteristic substrate activity relationships.

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† The abbreviations used are: LPC, 1-palmitoyl-sn-glycero-3-phosphocholine; BME, 2-mercaptoethanol; CK, creatine kinase; CMC, critical micelle concentration; FA, fatty acid; 14C-LPC, 1-(14C)-palmitoyl-sn-glycero-3-phosphocholine; HPLC, high performance liquid chromatography; PC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine.
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**EXPERIMENTAL PROCEDURES**

**Preparation of Cytosolic Extracts**—New Zealand White rabbits fed ad libitum were killed by cervical dislocation. Hearts were removed promptly and placed in homogenization medium (0.25 M sucrose, 10 mM phosphate, 1 mM EDTA, and 1 mM dithiothreitol, pH 7.40) at 0-4 °C. Ventricular muscle was washed extensively in buffer, minced to form a paste, passed through a Harvard Apparatus Co. tissue press, and homogenized with a Potter-Elvehjem apparatus (six strokes) to yield a 25% (w/v) homogenate. The homogenate was centrifuged sequentially at 3,000, 10,000, and 14,000 rpm for 10 min and the final supernatant centrifuged at 100,000 X g for 1 h. The mitochondrial fraction (10,000 X g supernatant) was decanted and used as cytosol extract. The microsomal pellet was washed and resuspended in homogenization medium. Cytosolic and microsomal preparations contained an average of 8 and 3 mg of protein per ml, respectively.

**Assay Systems**—Microsomal or cytosolic protein was added to 700 μl of 75 mM phosphate and 3 mM MgCl2 buffer, pH 7.4, containing 14C-LPC (specific activity approximately 10,000 dpm/nmol) and incubated in a metabolic shaker at 37 °C for 10 min. Butanol, 700 μl (pH 7.0, 0-4 °C), was added and the mixture vortexed twice for 10 s. Layers were separated by centrifugation and the organic phase stored in a sample vial. Butanol extraction resulted in nearly quantitative recovery of reaction products (FA, 98%; PC, 96%; and LPC, 89%) based on partition coefficient analysis, extracted over 90% of the total radioactivity from the aqueous phase (the remaining radioactivity in the fatty acid phase was >98% LPC as ascertained by TLC), and gave identical results when compared with conventional CHCl3/MeOH separations. Lipids were separated by HPLC or TLC or both. Each method gave the same results. HPLC separations utilized a cation exchange column (PXS-10/25-SCX, Whatman) as the stationary phase and acetonitrile/methanol/H2O (300:90:70) as the mobile phase.

**RESULTS**

**Partial Purification of Lysophospholipase and Lysophospholipase-Transacylase**—Twelve rabbits were killed by cervical dislocation. Cytosolic extracts were prepared as previously described except that three 30-s bursts of a Polytron homogenizer at one-half maximal setting were used instead of homogenization with an Elvehjem apparatus. To 175 ml of cytosol prepared from ventricular muscle, 65 g of solid ammonium sulfate were added over 2 min and stirred for an additional 9 min. The precipitate formed during this interval which did not contain lysophosphatidylcholine was removed by centrifugation at 100,000 X g for 5 min and quickly resuspended in 1 ml of 25 mM PO4, pH 7.50, 10% glycerol, and 10 mM BME. The protein was then dialyzed against 100 volumes of Buffer A for 14 h at 0 °C. A precipitate formed during this interval which did not contain lysophosphatidylcholine was removed by centrifugation at 100,000 X g for 15 min. The supernate was then loaded onto a DEAE Sephadex column (2.9 X 26 cm) previously equilibrated with Buffer A. After the UV absorbance had fallen to baseline, the column was developed with 10 volumes of a

![FIG. 1. Pathways of LPC metabolism. Top left, LPC acyltransferase; bottom left, lysophospholipase; horizontal reaction, lysophospholipase-transacylase.](http://www.jbc.org/Downloaded_from http://www.jbc.org)
linear NaCl gradient (0-600 mm) at a flow of 30 ml/h. The fractions containing lysophospholipase-transacylase activity eluted at approximately 400 mm NaCl and were subsequently pooled, dialyzed against 50 volumes of 28 mm potassium phosphate buffer (pH 7.0) containing 10% glycerol and 10 mm BME, for 12 h at 0 °C, and loaded onto a column (2.0 × 10 cm) of hydroxyapatite previously equilibrated with the same buffer. After elution of the void volume, transacylase activity was eluted with a linear gradient of 28-500 mm potassium phosphate, pH 7.0 (containing 10% glycerol and 10 mm BME), at a flow rate of 45 ml/h. Active fractions eluted at approximately 200 mm PO4, and were concentrated with an Amicon device with a YM-10 filter, from 45 ml-5 ml. The concentrated protein was loaded onto an ACA 44 column (92 × 1.6 cm) and gel filtration was conducted at a flow of 12 ml/h with 25 mm sodium phosphate buffer, pH 7.0, containing 10% glycerol, 10 mm BME and 0.5 mM NaCl. The active fractions were dialyzed against 100 volumes of sodium phosphate buffer (pH 7.0) containing 10% glycerol and 10 mm BME, for 10 h and loaded onto a polylysine agarose column (0.9 × 10 cm). A 0–700 mm NaCl gradient was run and active fractions eluted at approximately 150 mm NaCl.

**Source and Purity of Materials—** LPC was obtained from New England Nuclear and was >98% pure, ascertained by HFLC (16). DEAE-Sepacel was obtained from Pharmacia, polylysine agarose from Sigma, ACA 44 resin from LKB, and hydroxyapatite from Bio-Rad. C. adamanteus phospholipase A2 was obtained from Sigma.

**RESULTS**

Initial experiments identified the reaction products resulting from incubation of microsomal or cytosolic protein with [1-14C]-LPC. Incubations of microsomes with [1-14C]-LPC resulted in the release of fatty acid and synthesis of phosphatidylcholine with specific activities of 5.9 and 4.2 nmol/mg/min under conditions as shown in Table I. In addition, incubation of cytosolic protein with [1-14C]-LPC resulted in the release of fatty acid and synthesis of PC with specific activities of 1.1 and 0.4 nmol/mg/min. Neither the cytosolic nor the microsomal fractions demonstrated phospholipase activity when incubated with sonicated dispersions of 1,2-[1-14C]dipalmitoyl PC in phosphate buffer (pH 7.4). The mitochondrial fraction displayed the lowest activity for fatty acid release or PC synthesis (Table I) and consequently was not explored further. Radioactivity in PC synthesized by the microsomal or cytosolic proteins was not present in choline plasmalogens reflected by the observation that mild acid hydrolysis (0.1 N HCl in emulsions of butanol and H2O at 22 °C for 15 min) did not result in release of radiolabeled aldehydes and radioactivity was quantitatively recovered in PC. Furthermore, radioactivity was not present in glycerol ethers under these conditions since base-catalyzed methanolysis (1 N NaOH in MeOH at 22 °C for 15 min) of PC synthesized by microsomal or cytosolic protein resulted in quantitative production of radiolabeled FA methyl esters but no radioactivity co-migrating with LPC.

CK was used as a marker for cytosolic enzymes and was found to have a specific activity of 660 ± 3 IU/mg of protein (mean ± standard error, n = 6) in the cytosolic fraction but only 11.5 ± 0.4 IU/mg of protein (n = 6) in the microsomal fraction. No latent CK activity was detected in the microsomal fraction after preincubation in ethanol. Na-K ATPase had a specific activity of 7.8 ± 0.1 μmol/mg/h in the microsomal fraction but could not be detected in the cytosolic fraction (n = four of each). Since the microsomal fraction contained the highest enzymatic specific activity for fatty acid release as well as PC synthesis, membrane-bound enzymes in rabbit ventricular homogenates must hydrolyze as well as acylate LPC. The cytosolic fraction contains an enzyme(s) which hydrolyzes LPC and synthesizes PC which cannot be accounted for by microsomal contamination since the membrane marker Na-K ATPase was not detected in the cytosolic fraction. Since the mitochondrial fraction has the lowest specific activity for either PC synthesis or fatty acid release, contamination with mitochondrial components cannot account for any of the higher specific activities observed. These results demonstrate that the cytosol contains enzyme(s) that hydrolyze and acylate LPC which are separate and distinct from their microsomal counterparts. This view is substantiated by independent criteria delineating differences between the cytosolic and microsomal enzyme systems (see below).

**Acyl-CoA Dependence of PC Synthesis by Microsomal Enzymes and Cytosol Enzymes**—Microsomal PC synthesis required palmitoyl-CoA which could be replaced by ATP and CoA in combination but not by either alone (Table II). Thus, PC synthesis by microsomes is acyl-CoA dependent requiring the presence of a high energy thioester for enzymatic esterification. This interpretation is substantiated by the similar rates for incorporation of [1-14C]palmitoyl-CoA and [1-14C]-LPC into PC by microsomes (Table I). Among potential source(s) for FA in these incubations are: 1) endogenous FA in the microsomal preparation; 2) FA produced by enzymatic hydrolysis of LPC; and 3) FA produced by hydrolysis of endogenous lipids in the microsomal fraction during the incubation. The production of radiolabeled FA decreased by >90% in incubations containing both ATP and CoA, suggesting that possibility (2) is quantitatively most important.

Incubation of [1-14C]-LPC with cytosolic protein resulted in PC synthesis that was independent of the presence of acyl-CoA, CoA, ATP, or combinations of CoA and ATP (Table II). Thus, PC synthesis by the cytosolic fraction was acyl-CoA independent with similar rates of PC synthesis in the presence or absence of acyl-CoA or an acyl-CoA-generating system. In support of this interpretation was the marked difference in incorporation of [1-14C]palmitoyl-CoA or [1-14C]-LPC into PC when incubated with cytosolic protein (Table I). These results demonstrate that the cytosolic PC synthetic pathway is acyl-CoA independent and does not proceed by esterification of a high energy thioester.

**Positional Specificity of Acylation**—To further define possible differences between the acyl-CoA-dependent and the acyl-CoA-independent pathway, studies were performed to characterize the positional specificity of acylation of PC synthesis.

**Table I**

| Condition | Subcellular fraction | Label | Co-substrate | Vmax/E | Vmax/E |
|-----------|---------------------|-------|--------------|--------|--------|
| 1         | Microsomes          | [1-14C]-LPC (30) | Palm-CoA (100) | 5.9 ± 0.5 | 0.2 ± 0.1 |
| 2         | Microsomes          | [1-14C]-LPC (100) | LPC (100) | 1.1 ± 0.2 | 4.2 ± 0.3 |
| 3         | Microsomes          | [1-14C]-LPC (100) | | 4.9 ± 0.2 | |
| 4         | Cytosol             | [1-14C]-LPC (100) | LPC (100) | 1.1 ± 0.1 | 0.4 ± 0.1 |
| 5         | Cytosol             | [1-14C]-Palmitoyl-CoA (100) | | 0.06 ± 0.03 | |
| 6         | Mitochondria        | [1-14C]-LPC (100) | Palm-CoA (100) | 0.3 ± 0.1 | 0.2 ± 0.04 |
Acyl-CoA dependence of microsomal PC synthesis and independence of cytosolic PC synthesis

300 μg of microsomal protein or 800 μg of cytosolic protein were incubated at 37 °C for 15 min in pH 7.4 phosphate buffer with 100 μM 14C-LPC in a total volume of 1.0 ml. Reaction products were extracted into butanol and analyzed by HPLC as described under "Experimental Procedures." Results are means of four different determinations on two separate preparations. Palm-CoA = palmitoyl-CoA.

| Protein     | Palm-CoA | CoA | ATP | dpm PC |
|-------------|----------|-----|-----|--------|
| Microsomes  |          | 10  |     | 624    |
| Microsomes  | 100      |     |     | 11,527 |
| Microsomes  |          | 100 |     | 19,318 |
| Microsomes  |          | 100 | 100 | 676    |
| Microsomes  | 100      | 3   | 3   | 584    |
| Cytosol     | 100      |     |     | 21,228 |
| Cytosol     | 100      | 3   | 3   | 6,252  |
| Cytosol     | 100      |     | 3   | 6,235  |
| Cytosol     | 100      | 3   |     | 5,768  |
| Cytosol     | 100      | 3   |     | 6,244  |
| Cytosol     | 100      |     |     | 6,297  |
| Cytosol     | 100      |     | 3   | 6,481  |
| None        | 100      |     |     | 237    |

Microsomal protein (300 μg) was incubated with 100 μM of 14C-LPC and 100 μM palmitoyl-CoA or with 100 μM unlabeled palmitoyl-LPC and 100 μM [14C]palmitoyl-CoA for 15 min, extracted, and separated by HPLC. PC was treated with phospholipase A2, and reaction products separated as described under "Experimental Procedures." Cytosolic protein was incubated with 100 μM 14C-LPC alone or with 100 μM 14C-LPC and 100 μM palmitoyl-CoA. Results are expressed as the ratio of radioactivity in fatty acid divided by total released radioactive activity expressed as a percentage. Data are means ± S.E. of at least five determinations from a total of three preparations. Palm-CoA = palmitoyl-CoA.

Separation of Cytosolic LysoPhospholipase from LysoPhospholipase-Transacylase—Anion exchange chromatography separated cytosolic lyso phospholipase from lysophospholipase-transacylase (Fig. 2). Cytosolic lyso phospholipase eluted in the void volume and was completely devoid of PC synthetic activity. Lysophospholipase-transacylase eluted at approximately 400 mM sodium chloride and each active fraction had a 1:1:1 ratio of radioactivity in PC compared to FA. The active fractions were purified further by chromatography on hydroxyapatite. Lysophospholipase-transacylase activity eluted at approximately 200 mM potassium phosphate. Again the ratio of PC/FA radioactivity was 1:1:1. Active fractions were concentrated as described under "Experimental Procedures" and loaded onto an AcA 44 column. Lysophospholipase-transacylase activity eluted as a single peak with a 1:1:1 distribution of radioactivity in PC/FA. Active fractions were then dialyzed against pH 7 sodium phosphate buffer and loaded onto a polylysine agarose column. Lysophospholipase-transacylase eluted at 200 mM sodium chloride, again with a 1:1:1 ratio of radioactivity in PC/FA. Partially purified lysophospholipase-transacylase synthesized PC at a rate of 3.2 nmol/mg/min at a substrate concentration of 2 μM LPC. A summary of the purification is shown in Table IV.

Substrate Dependence—LPC acyltransferase had a maximum velocity of 5.1 nmol/mg/min with an apparent K_m for LPC of 14 μM and an apparent K_m for palmitoyl-CoA of 7 μM (Fig. 3). Microsomal lysophospholipase activity was dependent upon the ratio of substrate to microsomal protein (Fig. 4) with marked inhibition at high substrate/protein ratios. Additional experiments with 10 μg of microsomal protein revealed an inflection point at 3 μM LPC, well below published values of the CMC (22). The inflection point had a constant protein/substrate ratio of 3.3 ± 0.1 μg of protein/nmol of LPC (X ± S.E.). The maximum velocity at each protein concentration tested was similar (5.9 ± 0.5 nmol/mg/min (X ± S.E.), although it occurred over an order of magnitude variation in substrate concentration. Substrate dependence differed considerably for the cytosolic enzymes. Cytosolic lysophospholipase (partially purified fraction after DEAE-Sepharcl chromatography) had a maximum velocity of 9.5 nmol/mg/min and an apparent K_m for LPC of 7.5 μM (Fig. 5). Lysophospholipase-transacylase (partially purified after DEAE-Sepharcl chromatography) demonstrated Michaelis-Menten kinetics for fatty acid release with an apparent K_m of 14 μM. However, substrate dependence for PC synthesis was nonlinear, especially at low substrate concentrations (Fig. 6).
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Fig. 2. Purification of lysophospholipase-transacylase. The ammonium sulfate precipitate (0-60% saturation) was resuspended, dialyzed, and chromatographed on DEAE-Sephacel (A), hydroxylapatite (B), AcA 44 (C), and polylysine agarose (D) as detailed under "Experimental Procedures." Aliquots of fractions were incubated with \(^{14}\)C-LPC for 15 min, extracted with butanol, and analyzed by one-dimensional TLC. O, FA radioactivity; . . . . , absorbance at 280 nm; - - - , salt gradient.

Table IV
Summary of purification of lysophospholipase-transacylase from rabbit heart

| Step                  | Protein | Total activity | Specific activity | Purification factor | Yield (%) |
|-----------------------|---------|----------------|------------------|--------------------|-----------|
| Homogenate            | 5796    | 116            | 0.02             | 1                  | 100       |
| Cytosol               | 675     | 155            | 0.26             | 13                 | 100       |
| Ammonium sulfate      | 122     | 76             | 0.62             | 31                 | 65        |
| DEAE-Sephacel         | 54      | 44             | 0.80             | 41                 | 38        |
| Hydroxylapatite       | 1.2     | 31             | 7.3              | 367                | 27        |
| AcA 44                | 0.18    | 3              | 15.8             | 790                | 3         |
| Polysyline            | 0.02    | 2              | 97.7             | 4885               | 2         |
| Total                 |         |                |                  |                    |           |
|                       | 21523   | 15105          | 0.02             | 1.5                | 100       |

* One unit of activity is defined as the activity necessary to convert 2 nmol of LPC into 1 nmol of PC and 1 nmol of glycerophosphoryl choline in 1 min at 37 °C.

pH Dependence—Fatty acid release during incubations of microsomes with \(^{14}\)C-LPC had maximum activity from pH 7.0-8.0 but was markedly attenuated at pH 6.5 (rate = 19% of maximum, mean of three preparations). In addition, microsomal lysophospholipase activity at pH 6.0 was less than 10% that at pH 7.0 at every substrate and protein concentration shown in Fig. 4. Similar pH profiles were obtained with TrisCl buffer substituted for phosphate. In contrast, PC synthesis by microsomal protein showed a flat pH curve in the range of 6-8.5 when measured with either radiolabeled LPC and cold palmitoyl-CoA or radiolabeled palmitoyl-CoA and cold LPC (n = three of each). Cytosolic lysophospholipase had a broad pH optimum between 7 and 8 and was only mildly attenuated (rate = 80% of control) at pH 6.0 (n = 3). Cytosolic lysophospholipase-transacylase demonstrated a bell-shaped pH profile for both fatty acid release and PC synthesis with an optimum at pH 7.0 (n = 3).

Discussion

This investigation demonstrates the presence of four enzymes in rabbit ventricular homogenates which metabolize LPC. The microsomal fraction contained lysophospholipase activity which demonstrated unusual kinetics. The reaction velocity was not linearly related to the amount of protein in the incubation media. At a constant protein/concentration reaction velocity increased with increasing substrate concentration until an inflection point was reached when the inverse became true at substrate concentrations higher than the inflection point. The inflection point occurred at a constant protein/substrate ratio and was associated with a constant maximum velocity. The maximum velocity was independent of the total concentration of substrate in the incubation medium but was related to the substrate/protein ratio. This suggests that the relevant kinetic parameter is the density of LPC in the membrane that is potentially capable of interacting...
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**FIG. 4.** Lineweaver-Burk plot of microsomal fatty acid release. 10-200 \( \mu \text{M} \) \(^{14}\text{C}\)-LPC was incubated with microsomal protein (35-300 \( \mu \text{g} \)) for 10 min at 37 °C. Reaction products were extracted into butanol, separated by one-dimensional TLC, and analyzed by scintillation spectrometry. ○, 35 \( \mu \text{g} \); ▲, 75 \( \mu \text{g} \); △, 150 \( \mu \text{g} \); ●, 300 \( \mu \text{g} \) of microsomal protein. Results are means ± S. E. of three determinations.

**FIG. 5.** Lineweaver-Burk plot of cytosolic lysophospholipase activity. 40 \( \mu \text{g} \) of protein (from the void volume of the DEAE-Sephacel column) were incubated with from 5-200 \( \mu \text{M} \) \(^{14}\text{C}\)-LPC at 37 °C for 10 min. Reaction products were extracted into butanol, separated by TLC, and quantitated by scintillation spectrometry. Results are means ± S. E. of three determinations.

Lysoosphospholipase activity has previously been noted for rat brain lysophospholipase and has been interpreted as inhibition by micellar aggregates of LPC (22). Subsequently, the possibility that a critical substrate/protein ratio in the membrane is responsible for lysophospholipase inhibition has been suggested (23). Utilizing 16-doxyl stearate as a probe, we have noted marked alterations in the rotational correlation times of myocardial microsomal membranes exposed to LPC. Since inhibition of lysophospholipase activity occurs at submicellar concentrations of LPC, the inhibitory nature of “high” substrate concentrations can not be related solely to the presence of micellar aggregates of substrate but rather most likely reflects alterations in the biophysical characteristics of the membrane preparation produced by exceeding a critical mole proportion of LPC.

Myocardial ischemia is accompanied by an abrupt increase in hydrogen ion concentration to values approaching pH 6 (24). It is anticipated, therefore, that membrane bound lysophospholipase activity would be severely attenuated during myocardial ischemia potentially contributing to accumulation of LPC we have previously noted in ischemic tissue (4). The abrupt decline in activity during incubations with mild acidosis can not be related to titration of phosphate ion, since a similar profile was obtained with Tris-C1 buffer. Similarly, the decline in activity can not be related solely to altered inflection points with acidosis since the activity at pH 6.0 was <10% maximal activity over a 20-fold variation in substrate/protein ratios at four different protein concentrations.

* R. W. Gross, unpublished observations.
The microsomal fraction contained LPC acyltransferase exhibiting acyl-CoA-dependent PC synthesis, positional selectivity of acylation of the C-2 hydroxyl of LPC by the acyl substrate palmitoyl-CoA, and identical reaction velocities when either palmitoyl-CoA or LPC was used as the radiolabel. These characteristics as well as the $K_m$ for acyl-CoA and LPC are similar to those for LPC acyltransferase identified by others in rat liver microsomes (8). LPC acyltransferase has a broad pH profile and, thus, its activity would not be expected to be altered by myocardial ischemia on the basis of pH effects alone. Myocardial ischemia appears to result in an increase in cytosolic acyl-CoA (25) which could increase LPC metabolism by a mass action effect since the apparent $K_m$ for acyl-CoA is near the physiological concentration of this moiety.

Rabbit ventricular muscle contains enzymes in the cytosolic fraction which hydrolyze as well as transacylate LPC. Cytosolic lysophospholipase was present in the void volume after DEAE-Sephacel column chromatography and was unaccompanied by transacylase activity, thus demonstrating the presence of two functionally distinct enzymes in the cytosolic fraction. Cytosolic lysophospholipase was distinguished from microsomal lysophospholipase by: 1) its broad pH curve, 2) lack of substrate inhibition at high substrate/protein ratios, and 3) Michaelian kinetics with an apparent $K_n$ of 7.5 $\mu$M. No discontinuities were noted as a function of increasing concentrations (from monomeric to micellar) of substrate.

Lysophospholipase-transacylase was identified in the cytosolic fraction of rabbit ventricular homogenates by the presence of acyl-CoA-independent PC synthesis, equal distribution of labeled esterified FA at the C-1 and C-2 carbons of synthesized PC even in the presence of excess exogenous palmitoyl-CoA, and parallel pH profiles for FA and PC synthetic activity. Each fraction in the salt eluate of the DEAE-Sephacel column as well as in the hydroxylapatite, gel filtration, and poly-lysin chromatographies contained a 1:1:1 ratio of radioactivity in PC/FA, demonstrating co-purification of hydrolytic as well as transacylase activities. Thus, after a 4800-fold purification from crude homogenate, hydrolytic as well as transacylase activities co-migrated, lending support to the bifunctional nature of a single protein. Although fatty acid release obeyed Michaelis-Menten kinetics, double reciprocal plots of PC synthetic activity versus substrate concentrations were parabolic. The non-Michaelian kinetics of PC synthesis by lysophospholipase-transacylase has previously been noted for rat lung lysophospholipase-transacylase (11), and recently it has been suggested that transacylation of LPC occurs only when micellar substrate is present (12). Myocardial LPC transacylase differs from lysophospholipase-transacylase described in rat lung cytosol with respect to at least four functional features: 1) the ratio of radioactivity in PC/fatty acid is 1.1:1 after contaminating lysophospholipase is removed (contrasting with a 1:2.4 ratio for rat lung lysophospholipase-transacylase (11)); 2) myocardial lysophospholipase-transacylase reaches half-maximal velocities for PC synthesis at substrate concentrations more than 1 order of magnitude less than rat lung lysophospholipase-transacylase (11); 3) transacylase activity while non-Michaelian at submicellar concentrations of LPC is evident at the lowest substrate concentration examined (1.2 $\mu$M) well below published values of the CMC (22); and 4) myocardial lysophospholipase-transacylase possesses a broad pH curve with maximum activity at pH 7.0 compared to an optimum between pH 6.3 and 6.8 for rat lung lysophospholipase-transacylase (26). Judging from concentrations of LPC in myocardium (4), it appears that disaturated phosphatidylcholine in the heart can potentially be synthesized by lysophospholipase-transacylase. Investigations are currently in progress to ascertain whether LPC imbedded in phosphatidylcholine bilayers can be transacylated at significant rates.

The approach presented here results in a 4800-fold purification from crude homogenate with the partially purified enzyme able to transacylate submicellar concentrations of LPC (2 $\mu$M) and retain the comparatively high transacylase/lysophospholipase ratio present after DEAE-Sephacel chromatography. Although it is not yet certain that a single enzyme catalyzes both hydrolysis and transacylation in rabbit hearts (SDS-PAGE revealed three bands), the co-migration of hydrolytic and transacylase activities through every fraction of three successive chromatographic steps suggests that this is the case.

REFERENCES

1. Sobel, B. E., Corr P. B., Robison, A. K., Goldstein, R. A., Witkowski, F. X., and Klein, M. S. (1978) J. Clin. Invest. 62, 546-553
2. Corr, P. B., Snyder, D. W., Cain, M. E., Crafford, W. A., Jr., Gross, R. W., and Sobel, B. E. (1981) Circ. Res. 49, 264-263
3. Gross, R. W., Crafford, W. A., Jr., Sobel, B. E., and Corr, P. B. (1980) Circulation 62, Suppl. III, 280, (abstr.)
4. Corr, P. B., Saffitz, J. E., Lee, B. I., Gross, R. W., Keim, C., and Sobel, B. E. (1981) Circulation 64, Suppl. IV, 64, (abstr.)
5. Shapink, N. A., and Dowsws, E. (1981) Circ. Res. 49, 216-220
6. Snyder, D. W., Crafford, W. A., Jr., Glashow, J. L., Rankin, D., Sobel, B. E., and Corr, P. B. (1981) Am. J. Physiol. 241, 700-707
7. Gross, R. W., and Sobel, B. E. (1979) Trans. Assoc. Am. Physicians 92, 136-147
8. Lands, W. E. (1960) J. Biol. Chem. 235, 2223-2237
9. Van den Bosch, H., and De Jong, J. G. N. (1975) Biochim. Biophys. Acta 398, 244-257
10. Van Den Besselaa, A. M. H. P., Verheijen, J. H., and van den Bosch, H. (1976) Biochim. Biophys. Acta 431, 75-86
11. Brunley, E., and van den Bosch, H. (1977) J. Lipid Res. 18, 529-532
12. Van Heusden, G. P. H., Reulstofler, C. P. M., and Van den Bosch, H. (1981) Biochim. Biophys. Acta 683, 22-33
13. Leibovitz, Z., and Gatt, S. (1968) Biochim. Biophys. Acta 164, 394-431
14. Pettman, O. W., Illingworth, D. R., and Alexander, M. (1973) J. Neurochem. 20, 1659-1667
15. Chien, K. R., Abrams, J., Servoni, A., Martin, J. T., and Farber, J. L. (1978) J. Biol. Chem. 253, 4809-4817
16. Gross, R. W., and Sobel, B. E. (1980) J. Chromatogr. 197, 79-85
17. Bradford, M. (1976) Anal. Biochem. 72, 248-254
18. Wells, M. A., and Hanahbu, D. J. (1969) Methods Enzymol. 14, 178-184
19. Beller, G. A., Conroy, J., and Smith, T. W. (1976) J. Clin. Invest. 57, 441-456
20. Rosalki, S. B. (1967) J. Lab. Clin. Med. 69, 696-706
21. Van Heusden, G. P. H., and van den Bosch, H. (1979) Biochim. Biophys. Res. Commun. 90, 1000-1006
22. Leiboviz-BenGershon, Z., and Gatt, S. (1972) J. Biol. Chem. 247, 6840-6847
23. Leiboviz-BenGershon, Z., and Gatt, S. (1973) J. Biol. Chem. 249, 1525-1529
24. Hollis, D. P., Nunally, R. L., Taylor, G. J. IV, Weisfeldt, M. L., and Jacobus, W. E. (1978) J. Mag. Resonance 29, 319-330
25. Idell-Wenger, J. A., Groatjohann, L. W., and Neely, J. R. (1978) J. Biol. Chem. 253, 4310-4316
26. Viasen, G. M., and van den Bosch, H. (1978) Arch. Biochem. Biophys. 190, 373-384
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