A lysophospholipase was purified 506-fold from rat liver supernatant. The preparation gave a single 24-kDa protein band on SDS-polyacrylamide gel electrophoresis. The enzyme hydrolyzed lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylinositol, lysophosphatidylserine, and 1-oleoyl-2-acetyl-sn-glycero-3-phosphocholine at pH 6–8. The purified enzyme was used for the preparation of antibody and peptide sequencing. A cDNA clone was isolated by screening a λgt11 cDNA library with the antibody, followed by the selection of further extended clones from a λgt10 library. The isolated cDNA was 2,362 base pairs in length and contained an open reading frame encoding 230 amino acids with a Mr of 24,708. The deduced amino acid sequence showed significant similarity to Pseudomonas fluorescens esterase A and Spirulina platensis esterase. The three sequences contained the GXSXG consensus at similar positions. The transcript was found in various tissues with the following order of abundance: spleen, heart, kidney, brain, lung, stomach, and testis = liver. In contrast, the enzyme protein was abundant in the following order: testis, liver, kidney, heart, stomach, lung, brain, and spleen. Thus the mRNA abundance disagreed with the level of the enzyme protein in liver, testis, and spleen. When HL-60 cells were induced to differentiate into granulocytes with dimethyl sulfoxide, the 24-kDa lysophospholipase protein increased significantly, but the mRNA abundance remained essentially unchanged. Thus a posttranscriptional control mechanism is present for the regulation of 24-kDa lysophospholipase.

Lyso phospholipase hydrolyzes lysophosphatidylcholine (lyso-PC) to saturated fatty acid and sn-glycero-3-phosphocholine (GPC). This widely distributed enzyme contains a number of isoforms and is known to be regulated. The enzyme activity is modulated by lipid factors, such as acylcarnitine (1, 2), arachidonic acid (3, 4), and phosphatidic acid (4). An isoform of the enzyme was reported to be inducible in HL-60 cells during granulocyte differentiation (5, 6). The physiological roles of lysophospholipase have not been fully elucidated. A plausible function is the control of the intracellular level of the physiologically active lipid (7–17), lyso-PC.

Lysophospholipase were purified from various sources, such as beef pancreas (18), beef liver (19), rabbit myocardium (1, 2), human eosinophils (20), macrophage cell lines, P388D1 (3) and WEHI 265.1 (21), HL-60 cells (6), rat liver (4), and pig gastric mucosa (22). Lysophospholipase can be classified into two large groups according to their molecular masses (23). Large form enzymes (60 kDa) generally exhibit not only hydrolytic activity, but also transacylase activity, whereas small form enzymes (16.5–28 kDa) show only hydrolytic activity. Liver (19), heart (1, 2), and stomach (22, 24) contain both forms of enzyme. Furthermore, heart (1), macrophages (3, 21), HL-60 (6), and stomach (22) contain two small form enzymes. Sunaga et al. (22) raised antibody against each of the two small form enzymes (22 and 23 kDa) purified from pig gastric mucosa and showed that the antibodies did not cross-react. Hence, the two small form enzymes of stomach are immunologically different. Furthermore, the major, 22-kDa enzyme (gastric enzyme I) hydrolyzed lyso-PC, lysophosphatidylethanolamine (lyso-PE), lysophosphatidylinositol (lyso-PI), lysophosphatidylserine (lyso-PS). In contrast, the minor, 23-kDa enzyme (gastric enzyme II) hydrolyzed only lyso-PC and lyso-PE. Thus the two enzymes show distinct substrate specificities.

Little is known about the molecular structures of lysophospholipases except for pancreatic and eosinophilic enzymes (25, 26). Since the latter two enzymes have considerably different properties from other lysophospholipases, it is important to clone cDNAs for other lysophospholipases. In this paper, we report the purification and cDNA cloning of a small form lysophospholipase from rat liver. The enzyme had a molecular mass of 24 kDa on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and catalyzed the hydrolysis of a rather broad range of lysophospholipids, such as lyso-PC, lyso-PE, lyso-PI, and lyso-PS. The enzyme resembled pig gastric lysophospholipase I (22), bovine liver lysophospholipase I (19), and rabbit myocardial 23-kDa lysophospholipase (1). The cloned cDNA encoded 230 amino acids with a calculated molecular mass of 24,708 Da. The deduced amino acid sequence showed significant sequence similarity to microbial esterases and contained the GXSXG consensus conserved in the active site of serine-proglycero-3-phosphoethanolamine; GP(3)-glycero-3-phosphoserine; GPE (sn-glycero-3-phospho-myosinositol; PAGE, polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography; IPTG, isopropyl-β-D-thiogalactopyranoside.
proteases, lipases, and esterases (27). We also report the post-transcriptional control of the enzyme.

**Experimental Procedures**

**Materials**—1-[^14]C]palmitoyl-GPC (55 mCi/mmol), [α-^32]PdCTP, and [α-^32]PUTP were from Amersham International (Amersham, United Kingdom) (U.K.). 1-palmitoyl-sn-glycero-3-phosphate (1-palmitoyl-GP) and 1-palmitoyl-sn-glycerol were from Serdary Research Laboratories (London, U.K.). 1-Palmitoyl-sn-glycero-3-phosphoethanolamine (1-palmitoyl-GPE), 1-oleoyl-GPE, 1-acetyl-sn-glycero-3-phosphorothioate (1-acetyl-GSPE), 1-oleoyl-sn-glycerol-3-phosphate (1-oleoyl-GP), 1-oleoyl-2-acetylglycerol, 1-oleoyl-2-arachidonylglycerol, and 1-palmitoyl-2-oleoyl-sn-glycerol were purchased from Avanti Polar Lipids (Alabaster, AL). 1-Oleoyl-2-acetyl-3-phosphatidylethanolamine were obtained from Wako (Osaka, Japan). DEAE-Cellulofine A-500 was from Seikagaku Kogyo (Tokyo, Japan). Octyl-Sepharose CL-6B, Sephacryl S-200, Sephacryl S-300, and blue-Sepharose CL-4B were products of Pharmacia Fine Chemicals (Uppsala, Sweden). Protein Pak G-buty1 column used for high performance liquid chromatography (HPLC) was obtained from Millipore (Milford, MA).

**Lysophospholipase Assays**—The isotopic assay was performed for 20 or 60 min at 37°C in a 0.1-ml reaction mixture containing 20 mM Tris-HCl, pH 8.0, 0.4 mM 1[^14]C]palmitoyl-GPC (575 dpm/nmol), and enzyme as described previously (4). The colorimetric assay was performed for 20 min as described previously (4) except that 20 mM Tris-HCl, pH 8.0, and the NEFA-HR free fatty acid assay kit (Wako, Tokyo, Japan) were used instead of sodium phosphate, pH 6.0, and the Detamina NEFA 755 assay kit, respectively.

**Enzyme Purification**—All procedures were carried out at 4°C. Livers (95 g) from 12 male Wistar rats weighing about 250 g (Mai Animal Farm, Saitama, Japan), minced, and homogenized in 2 volumes of 0.25 M sucrose in solution A (20 mM Tris-HCl, pH 7.5, 0.1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 2 μg/ml antipain, 1 μg/ml chymotrypsin, 10 μg/ml benzamidine, 1 mM EDTA, 2 mM β-mercaptoethanol, 1 mM dithiothreitol, and 10% (v/v) glycerol) with a Teflon homogenizer. The homogenate was centrifuged at 18,000 g, and the supernatant was centrifuged at 100,000 g for 90 min. The resulting supernatant (115 ml) was applied to a DEAE-Cellulofine A-500 column (3.5 × 20 cm) equilibrated with solution A. The enzyme was eluted with a 540-ml linear gradient of NaCl (0–1.0 M) in solution A. Active fractions were combined, adjusted to 0.9 M NaCl and 0.01% (w/v) Triton X-100, and loaded onto an octyl-Sepharose CL-4B column (1.5 × 14 cm) equilibrated with solution A containing 0.9 M NaCl and 0.01% Triton X-100. The column was washed with 3 volumes of the same buffer and eluted with a linear gradient made from 75 ml of solution A containing 0.9 M NaCl and 0.01% Triton X-100 and 75 ml of solution A containing 20% (v/v) ethylene glycol and 0.5% Triton X-100. To the pooled eluate was added solid ammonium sulfate to bring its final concentration to 60% saturation. The mixture was centrifuged at 100,000 g for 90 min. The precipitate was dissolved in 3.7 ml of solution A containing 50% formamide, and then applied to a nitrocellulose membrane (Hybond-C, Amersham). The membrane was washed with 0.1% (w/v) SDS at 4°C overnight, washed, and then treated with a nitrocellulose solution. The filters were treated with the antiserum and then goat anti-rabbit IgG-alkaline phosphatase conjugate, followed by staining with the Protoblot Immunoscreening System (Promega). Positive plaques were rescued from the master plate, purified, and then amplified in Y10900r. The insert was excised from the plasmid and subcloned into pBluescript II KS+ (Stratagene). SG1-1 thus obtained was linearized and used for the preparation of radiolabeled RNA probe with the T7 RNA synthesis set (Nippon gene) and [α-^32]PUTP. The αgt10 cDNA library was hybridized with the RNA probe on Hybond-N filters (Amersham) in a solution containing 48% (v/v) formamide, 4.8 × 0.72 M NaCl, 0.072 M sodium citrate, pH 7.0, 1× Denhardt’s solution (30), 10% (w/v) dextran sulfate, and 0.2% SDS at 42°C overnight. The filters were washed with 2× SSC containing 0.1% SDS and twice with 0.2× SSC containing 0.1% SDS for 15 min at 65°C and then exposed to x-ray films (Fuji Photo Film, Kanagawa, Japan). Phages exhibiting positive signals were saved and analyzed.

**Sequence Analysis**—Both strands of DNA was sequenced by the dideoxy chain termination method (31) using the BcaBEST dideoxy sequencing kit (Takara, Tokyo) and [α-^32]PdCTP after subcloning into M13mp19. Peptides obtained by digestion of the enzyme with lysyl endopeptidase (1:100, by mass) were purified on a Chemco 3C 18 column (Chemco, Tokyo), and their sequences were determined as described by Hayashi et al. (32) using a 477A gas-phase protein sequence analyzer equipped with a 120A on-line phenylthiohydantoin analyzer (Biosystems, Tokyo).

**Expression of Lysophospholipase cDNA in E. coli Cells**—SG1-1 was cleaved at the NolI (positions 8 to 1) and SpeI (positions 982–987) sites, and the resulting fragment was cloned into pBluescript II SK− (Stratagene) which had been cleaved with NolI and SpeI. The resulting plasmid pSG1-1-Ex was transformed into E. coli XL1-Blue. The transformant was cultured in 10 ml of LB medium containing 50 μg/ml ampicillin and 40 μg/ml tetracycline for 150 min and then for 6 h in the presence of 10 μM IPTG. The cells were harvested, suspended in 1 ml of ice-cold lysis buffer (25 mM Hepes-NaOH, pH 7.6, 100 mM KCl, 0.1 mM EDTA, pH 8.0, 12.5 mM MgCl2, 0.1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 μg/ml chymotrypsin, 2 μg/ml benzamidine, 10 μg/ml bacitracin, 0.1% dithiothreitol, and 10% (v/v) glycerol), disrupted by sonication, and then subjected to enzyme assays and immunoblot analysis.

**Northern Blot Analysis**—Poly(A)+ RNA was subjected to electrophoresis in a formaldehyde-containing 1.2% agarose gel and transferred to a nitrocellulose membrane (Hybond-C, Amersham). The membrane was hybridized with [α-^32]PdCTP in a solution containing 25 mM potassium phosphate, pH 7.4, 5× SSC, 5× Denhardt’s solution, 50 μg/ml salmon sperm DNA, 50% formamide, and 10% dextran sulfate at 42°C. The membrane was washed twice with 1× SSC containing 0.1% SDS and twice with 0.25× SSC containing 0.1% SDS at room temperature for 15 min and then exposed to x-ray film. The hybridization intensity was quantified using a LKB 2202 ultrasonic laser densitometer.

**Cell Culture**—HL-60 cell line provided by the Japanese Cancer Re-
search Resources Bank was grown in Iscove's modified Dulbecco's medium (Life Technologies, Inc.) supplemented with 10% (v/v) fetal calf serum (Commonwealth Serum Laboratories, Melbourne, Australia). Terminal differentiation was achieved by incubating the cells with 1.25% (v/v) Me2SO for 6 days. Cells (2.5 × 10^7) were harvested by centrifugation at 200 × g and then resuspended in 300 µl of ice-cold lysis buffer as above and kept at −70 °C. Part of the cell suspension was sonicated and centrifuged at 10,000 × g for 20 min. The resulting pellet was resuspended in the same iced lysis buffer and used for enzyme assays and immunoblot analysis. The rest of the cell suspension was mixed with 1 ml of Isogen and used for the preparation of total RNA.

RESULTS

Purification of a Small Form Lysophospholipase from Rat Liver Supernatant—The liver tissue contains multiple lysophospholipase isozymes (4, 19). Rat liver supernatant was chromatographed on DEAE-Cellulofine A-500 at pH 7.5 using a NaCl gradient. Essentially all lysophospholipase activity was adsorbed to the column and eluted in three peaks when assayed at pH 7.5 using a NaCl gradient. Essentially all lysophospholipase activity was adsorbed to the column and eluted in three peaks when assayed at pH 8.0. Enzymes contained in individual peaks were immunologically distinguishable from each other. The second peak eluted with 0.35–0.45 M NaCl contained the largest activity. The third peak contained 60-kDa lysophospholipase-transacylase (4). We decided to characterize the second peak enzyme and clone the cDNA.

The second peak fractions were collected, adjusted to 0.9 M NaCl, and loaded onto an octyl-Sepharose column. The enzyme was eluted with a gradient of decreasing NaCl concentration and increasing ethylene glycol concentrations. The eluate was condensed by ammonium sulfate precipitation and passed through Sephacryl S-300 in the presence of Triton X-100. This step was very effective, resulting in more than 20-fold purification. The enzyme was further purified using blue-Sepharose, G-butyl HPLC, and then Sephacryl S-200. The overall purification of the enzyme was 506-fold with a yield of 9.6% (Table I). The use of Triton X-100 was needed to avoid aggregation of the enzyme. Glycerol and β-mercaptoethanol stabilized the enzyme. The enzyme was stored at −80 °C for at least 6 months without an appreciable loss of activity. The final enzyme preparation gave a single protein band on SDS-polyacrylamide gel electrophoresis with a molecular mass of 24 kDa (Fig. 1A). The molecular mass of the native enzyme was estimated to be 25 kDa on the Sephacryl S-200 column. Thus the enzyme appeared to be a monomeric protein. The final specific activity was 0.87 µmol/min/mg. This was increased to 2 µmol/min/mg by the addition of 0.4% (w/v) albumin to the assay. The N terminus of the enzyme was found to be blocked.

Properties of the Enzyme—The activity of the enzyme was not affected by Ca^2+ or Mg^2+ or EDTA. The enzyme was active in a wide pH range from 5.5 to 9.0. When examined with 1-palmitoyl-GPC, the substrate dependence curve was sigmoidal, and the double-reciprocal plot was upward concave (data not shown) as observed with other lysophospholipases (3, 4, 22, 33, 34). The data were fit to the Hill equation with a Hill coefficient of 1.25. K_M and V_MAX were calculated to be 0.17 mM and 1.55 µmol/min/mg.

We next examined the substrate specificity of the enzyme using a substrate concentration (0.4 µM) over 2 times higher than the K_M value determined for 1-palmitoyl-GPC. For limited availability of labeled substrates, we used the previously developed calorimetric assay method (4) with some modifications to improve sensitivity. As shown in Table II, the enzyme utilized various lysophospholipids, including lyso-PC, lyso-PE (1-oleoyl-GPE), lyso-PS, and lyso-PI. The acyl analog of platelet activating factor, 1-oleoyl-2-acetyl-PC, was also a good substrate. Lysophosphatidic acid and diacylphospholipids were poor substrates. The enzyme showed little lipase and general esterase activities.

Tissue Distribution of the Enzyme—Polyclonal antibody was raised against purified enzyme in a rabbit and used to examine the tissue distribution of the enzyme by immunoblot analysis

![Fig. 1. SDS-polyacrylamide gel electrophoresis, tissue distribution, and antibody cross-reactivity.](image-url)

**Table I**

| Fraction         | Protein (mg) | Specific activity (nmol/min/mg) | Total activity (nmol/min) | Purification (fold) | Yield (%) |
|------------------|--------------|--------------------------------|---------------------------|---------------------|----------|
| Supernatant      | 1600         | 1.72                           | 2750                      | 1                   | 100      |
| DEAE-Cellulofine | 570          | 1.59                           | 906                       | 0.92                | 33       |
| Octyl-Sepharose  | 57.9         | 11.2                           | 648                       | 6.51                | 24       |
| Sephacryl S-300  | 2.13         | 199                            | 424                       | 116                 | 15       |
| Blue-Sepharose   | 0.73         | 682                            | 498                       | 397                 | 18       |
| Protein Pak G-butyl | 0.56     | 771                            | 432                       | 448                 | 16       |
| Sephacryl S-200  | 0.31         | 870                            | 270                       | 506                 | 9.8      |

2 H. Sugimoto, unpublished observations.
All of the tissue supernatants examined contained the enzyme, but the intensity of the band densitometrically determined differed from tissue to tissue with the following order (arbitrary unit): testis, 0.96; liver, 0.85; kidney, 0.58; heart, 0.56; stomach, 0.53; lung, 0.20; brain 0.18; spleen, 0.10 (lanes 2–9). The antibody reacted with purified pig gastric lysophospholipase I, but not II (Fig. 1C). Consistently, antibody directed to pig gastric lysophospholipase I recognized the present enzyme (22). Thus the present enzyme is closely related to gastric lysophospholipase I.

Cloning and Nucleotide Sequence of 24-kDa Lysophospholipase cDNA—Screening for lysophospholipase cDNA was carried out in two steps. Plaque replicas (1 × 10^6 plaques) from a rat liver gt11 library were treated with the antibody and then goat anti-rabbit IgG alkaline phosphatase conjugate, followed by staining with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. A positive clone obtained was inserted into Bluescript KS(+) and the resulting plasmid, SG1-1, was used for the preparation of a radiolabeled RNA probe. By screening a gt10 library (1 × 10^6) with the RNA probe, we obtained clone SG2-1 with further extended 5’ and 3’ ends and poly(A)尾巴 (Fig. 2, upper panel). We determined the nucleotide sequences of SG1-1 and SG2-1 by the dideoxy chain termination method (31). The 2,362-basepair sequence of cDNA is shown in the lower panel. The initial isolate, SG1-1, contained 2,254 nucleotides from positions 78 to 2,331. Within the sequence there is an open reading frame at nucleotide positions 73–762 encoding 230 amino acid residues with a calculated molecular mass of 24,708 Da, followed by 1,597-base pair untranslated region with a poly(A)尾巴. We assumed the ATG codon at positions 73–75 as the translational initiation codon for the following reasons. First, the calculated molecular mass of the deduced amino acid sequence was consistent with the size of the enzyme. Second, when the open reading frame was expressed in E. coli cells, lyso-PC-hydrolyzing activity increased greatly (see below). The deduced amino acid sequence contained both of the two peptide sequences determined for the lysine endopeptidase-digested enzyme with a single amino acid mismatch at amino acid position 23 (Ala versus Ser). Although a low concentration of Triton X-100 was required for enzyme purification, Kyte and Doolittle’s hydrophathy analysis (35) predicted the protein to be rather hydrophilic. Chou and Fasman’s second structure analysis (36) suggested that the enzyme is composed of two α/β structures (37). The 3’-untranslated region contained two putative polyadenylation signals, ATTAAA (38, 39) and AATAAA (40) at positions 1,861 and 2,335, respectively.

Sequence Similarity to Pseudomonas fluorescens Carboxylesterase and Spirulina platensis Esterase—We searched the protein database for similar sequences using the FASTA program (41). P. fluorescens carboxylesterase (42) and S. platensis esterase (43) were found to bear significant sequence similarity to the enzyme (Fig. 3). The enzyme showed 34.0% identity to P.
cDNA encoded amino acid positions 3–230 as a plasmid, SG1-1-Ex, containing nucleotide positions 78–987 of obtained was cloned into pBluescript II SK(+) and subjected to transformant and thus assigned to be SG1-1-Ex-encoded. The three sequences contained the G consensus is fairly well correlated with the level of the enzyme protein

The enzyme (β-galactosidase) was the control. These re-

Expression of Lysophospholipase cDNA in E. coli Cells—SG1-1 was cleaved with NotI and SpeI, and the fragment obtained was cloned into pBluescript II SK(−). The resulting plasmid, SG1-1-Ex, containing nucleotide positions 78–987 of cDNA encoded amino acid positions 3–230 as a β-galactosidase fusion. The plasmid was introduced into E. coli XL1-Blue, and the transformant was treated with IPTG to express the fusion protein. Cells were disrupted by sonication and the extract was assayed for lysophospholipase activity using lysy-PC as the substrate. As shown in Fig. 4A, the transformant exhibited 10 times higher activity than the control cells. Immunoblot analysis was performed to examine whether the lysophospholipase protein was indeed present in the transformant. As shown in Fig. 4B, the transformant exhibited three bands, two of which were also seen in the vector control. The middle band with a molecular size slightly larger than purified enzyme was specific to the transformant and thus assigned to be SG1-1-Ex-encoded. The increase in size was consistent with the fusion of the N-terminal region of β-galactosidase to the enzyme. These results indicate that the cloned cDNA indeed encodes 24-kDa lysophospholipase. The deduced amino acid sequence of the enzyme (LYS) is compared with those of P. fluorescens esterase A (PSE) and S. platensis esterase (SPE). The numbers indicate the amino acid positions. Thick letters indicate the identical amino acids. The GXSXG consensus is 20.6% identity to S. platensis esterase in 210 amino acid overlap. The three sequences contained the GXSXG motif (27) at similar positions. However, there was no significant sequence similarity to the previously cloned pancreatic lysophospholipase (25) and eicosanophilic lysophospholipase (Charcot-Leyden crystal protein) (26) as examined by the LFASTA program (41).

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Hepatic Lysophospholipase cDNA

Figure 6. Induction of 24-kDa lysophospholipase in HL-60 cells by Me\textsubscript{2}SO. A, enzyme activity. The 10,000 \times g pellet (71 mg of protein) of HL-60 cells cultured for 6 days without (lane 1) or with Me\textsubscript{2}SO (lane 2) were assayed for lysophospholipase activity as described under “Experimental Procedures.” B, immunoblot analysis. The 10,000 \times g pellet (71 mg of protein) of HL-60 cells cultured without (lane 1) or with Me\textsubscript{2}SO (lane 2) and 100 ng of purified 24-kDa lysophospholipase (lane 3) were subjected to immunoblot analysis using anti-lysophospholipase antiserum. The arrows indicate the locations of molecular mass markers: carbonic anhydrase, 41.9 kDa; trypsin inhibitor, 32 kDa; lysozyme, 17.9 kDa. C, Northern blot analysis. Total RNA (10 \mu g) prepared from HL-60 cells cultured without (lane 1) or with Me\textsubscript{2}SO (lane 2) were subjected to Northern blot analysis using the cDNA probe as described under “Experimental Procedures” (upper panel). Arrows indicate the locations of 28 and 18 S rRNA. The blot was also probed with \beta-actin cDNA as the control (lower panel).

differentiation. After culturing for 6 days, morphological changes were noted. The cells were harvested and lysophospholipase activity was determined. Activity was mainly localized in the 10,000 \times g pellet. In agreement with the previous investigations (5, 6), lysophospholipase activity increased more than 3-fold by the Me\textsubscript{2}SO treatment (Fig. 6A). Consistently, the antibody against the 24-kDa lysophospholipase clearly recognized a 24 kDa-protein in Me\textsubscript{2}SO-treated HL-60 cells (Fig. 6B). Although its intensity was greatly increased by Me\textsubscript{2}SO treatment, the mRNA level remained essentially unchanged (Fig. 6C). These results clearly show that 24-kDa lysophospholipase is the same enzyme as the “Peak 2” lysophospholipase that was reported to be induced in differentiated HL-60 cells (6). Since the increase in the enzyme amount is not associated with the change in mRNA abundance, the induction is due to a post-transcriptional mechanism. Probably, stimulation of translation or stabilization of the enzyme protein is involved.

**DISCUSSION**

In the present study, we purified a small form lysophospholipase from rat liver and cloned its cDNA. It is interesting to compare this lysophospholipase with previously reported small form lysophospholipases. At least two types of small form lysophospholipase occur in mammalian tissues. Sunaga et al. (22) purified two small form lysophospholipases from pig gastric mucosa and examined their substrate specificities. The major, 22-kDa enzyme (gastric enzyme I) was able to hydrolyze lyso-PC, lyso-PE, lyso-PO, lyso-PS, and the analog of platelet-activating factor, 1-acetyl-2-acyl-GPC. In contrast, the minor, 23-kDa enzyme (gastric enzyme II) hydrolyzed only lyso-PC and lyso-PE. The hepatic 24-kDa enzyme shows similar substrate specificity to gastric enzyme I. Its antibody reacted with gastric enzyme I, but not II. Consistently, the hepatic enzyme was recognized by anti-gastric enzyme I, but not by anti-gastric enzyme II. Thus this enzyme is concluded to be the same enzyme as gastric enzyme I. The present enzyme has a similar molecular weight to bovine liver lysophospholipase I reported by de Jong et al. (19). The bovine enzyme I was shown to be active at pH 6–8 and efficiently hydrolyze 1-acetyl-2-acyl-GPC (44). Thus the present enzyme resembles bovine liver lysophospholipase I. Furthermore, immunoblot analysis indicated that the present enzyme is abundant in the heart tissue. Probably, the enzyme is also closely related to the 25-kDa lysophospholipase reported by Gross and Sobel (1). Only a few papers have dealt with successful cloning of lysophospholipase cDNA except for cDNAs encoding pancreatic lysophospholipase (25) and eosinophilic lysophospholipase (26). However, a sequence comparison revealed no significant sequence similarity between the present enzyme and these two lysophospholipases. The pancreatic enzyme cloned by Han et al. (25) is a secretory digestive enzyme and was later shown to be the same enzyme as cholesterol esterase by cDNA cloning (45). The present results support the previous proposal that pancreatic lysophospholipase should be called carboxyl ester lipase (46, 47). Eosinophilic lysophospholipase (Charcot-Leyden crystal protein) was predicted to have a molecular mass of 16.5 kDa from its cDNA (26). This size is exceptionally small among lysophospholipases. The final specific activity was reported to be very low, 23.4 nmol/h/mg (20). Thus pancreatic and eosinophilic enzymes are thought to be evolutionarily apart from other lysophospholipases. This idea would be strengthened by future cloning of other intracellular lysophospholipases, such as 60-kDa lysophospholipase-transacylase (4) and 23-kDa gastric lysophospholipase II (22).

It was shown that arachidonic acid release and lysophospholipase activity increased in differentiated HL-60 cells (5, 48, 49). One plausible function of the induction of lysophospholipase would be the removal of lyso-PC that is cytotoxic when accumulated (50). However, this may not be the sole function of lysophospholipase. Simultaneous induction of phospholipase A\textsubscript{2} and lysophospholipase would cause an increase in not only unsaturated fatty acid, but also saturated fatty acid. It is interesting to speculate that not only unsaturated fatty acid (51), but also saturated fatty acid might be needed for differentiated HL-60 cells. Garsetti et al. (6) showed that the increase of lysophospholipase activity in HL-60 was due to the expression of a single lysophospholipase isoform. The present study has extended their observations and clearly identified the induced enzyme as 24-kDa lysophospholipase. Furthermore, the enzyme induction was shown to involve posttranscriptional mechanism, probably stimulation of translation or stabilization of the enzyme. These results would be an important clue to the full understanding of the control and physiological roles of this enzyme.

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