Cloning and Expression of cDNA Encoding Rat Liver 60-kDa Lysophospholipase Containing an Asparaginase-like Region and Ankyrin Repeat*

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Mammalian tissues contain small form and large form lysophospholipases. Here we report the cloning, sequence, and expression of cDNA encoding the latter form of lysophospholipase using antibody raised against the enzyme purified from rat liver supernatant (Sugimoto, H., and Yamashita, S. (1994) J. Biol. Chem. 269, 6252–6258). The 2,539-base pair cDNA encoded 564 amino acid residues with a calculated Mr of 60,794. The amino-terminal two-thirds of the deduced amino acid sequence significantly resembled Escherichia coli asparaginase I with the putative asparaginase catalytic triad Thr-Asp-Lys and was followed by leucine zipper motif. The carboxyl-terminal region carried ankyrin repeat. When the cDNA was transfected into HEK293 cells, not only lysophospholipase activity but also asparaginase and platelet-activating factor acetylhydrolase activities were expressed. Reverse transcription-polymerase chain reaction revealed that the transcript occurred at high levels in liver and kidney but was hardly detectable in lung and heart from which large form lysophospholipases had been purified, suggesting the presence of multiple forms of large form lysophospholipase in mammalian tissues.

A major fate of lysophosphatidylcholine (lyso-PC)1 generated by the action of phospholipase A2 on membrane phosphatidylcholine (PC) is the degradation to fatty acid and sn-glycero-3-phosphorylcholine (GPC). Although several types of enzymes have been shown to be able to catalyze this reaction, including cytosolic phospholipase A2 (1–4), calcium-independent phospholipase A2 (5, 6), and carboxy ester lipase (pancreatic lysophospholipase) (7–9), mammalian tissues contain specific lysophospholipases, which is believed to play a major role in the hydrolytic degradation of lyso-PC.

Mammalian lysophospholipases can be classified into small form and large form enzymes according to their molecular size (10). Small form lysophospholipases were purified from beef liver (25 kDa) (11), rat liver (24 kDa) (12), rabbit myocardium (22 and 23 kDa) (13), pig gastric mucosa (22 and 23 kDa) (14), macrophage cell lines (27 and 28 kDa) (15, 16), HL-60 cells (20 and 21 kDa) (17), and Charcot-Leyden crystal protein (17.4 kDa) (18). Some tissues were found to contain two chromatographically or immunologically distinguishable small form lysophospholipases with slightly different molecular masses (13–17, 19). cDNAs for small form lysophospholipase were recently cloned from rat liver (12) and P388D1 murine macrophages (20). The rat liver enzyme comprised 230 amino acids with a calculated molecular mass of 24,708 Da (12) and showed significant similarity to microbial esterases (21, 22) with the GX-XG consensus conserved in the active sites of serine esterases, proteases, and lipases. The murine macrophage enzyme was also composed of 230 amino acid residues and showed 96.5% amino acid identity to the rat liver enzyme (20). Its activity was lost when the Ser119 in the conserved G consensus was changed to Ala by site-directed mutagenesis. The sequences of the liver and macrophage enzymes showed no sequence similarity to other lysophospholipid-hydrolyzing enzymes, such as carboxyl ester lipase (7, 8) and Charcot-Leyden crystal protein (23).

Large form lysophospholipases with molecular masses of 50–63 kDa were purified from beef liver (60 kDa) (11), rabbit myocardium (63 kDa) (24), and rat liver (60 kDa) (25). Partially purified enzymes were also obtained from lung (50 kDa) (26) and stomach (27). Most lysophospholipases of this type were shown to exhibit transacylase activity as well, producing one molecule of disaturated phosphatidylcholine from two molecules of lyso-PC releasing GPC. In addition, the beef liver enzyme was reported to exhibit platelet-activating factor (PAF) acetylhydrolase activity (28). However, the molecular structure of the large form lysophospholipase was totally unknown. In this paper, we report the cDNA cloning of a large form lysophospholipase from rat liver using antibody against the purified enzyme (25). The cloned cDNA encoded 564 amino acids with a calculated molecular mass of 60,794 and showed no sequence similarity to the small form lysophosphatidylcholine from two molecules of lyso-PC releasing GPC. In addition, the beef liver enzyme was reported to exhibit transacylase activity as well, producing one molecule of disaturated phosphatidylcholine from two molecules of lyso-PC releasing GPC. In addition, the beef liver enzyme was reported to exhibit platelet-activating factor (PAF) acetylhydrolase activity (28). However, the molecular structure of the large form lysophospholipase was totally unknown. In this paper, we report the cDNA cloning of a large form lysophospholipase from rat liver using antibody against the purified enzyme (25). The cloned cDNA encoded 564 amino acids with a calculated molecular mass of 60,794 and showed no sequence similarity to the small form lysophospholipases of rat liver and macrophage, indicating that the large form lysophospholipase is evolutionarily apart from the small form enzyme.

The large form enzyme was predicted to be composed of two domains, the amino-terminal putative catalytic domain significantly resembling Escherichia coli asparaginase I and the carboxyl-terminal ankyrin repeat. When the cDNA was transfected into HEK293 cells, not only lysophospholipase activity but also asparaginase and PAF acetylhydrolase activities were expressed.

EXPERIMENTAL PROCEDURES

Materials—1-[14C]Palmitoyl-GPC (55 mCi/mmol), [α-32P]dCTP, and [α-32P]UTP were obtained from Amersham Pharmacia Biotech. 1-Hexadecyl-2-acetyl-sn-glycero-3-phospho-[14C]choline (55 mCi/mmol) and

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2. The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EMBL Data Bank with accession number(s) AB009372.

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4. The abbreviations used are: lyso-PC, lysophosphatidylcholine; PC, phosphatidylcholine; GPC, sn-glycero-3-phosphorylcholine; PAF, platelet-activating factor; PCR, polymerase chain reaction; RT-PCR, reverse transcription-PCR.
Lysophospholipase cDNA

1-[3H]Asparagine (250 mCi/nmol) were from American Radiolabeled Chemicals, Inc. (St. Louis, MO). 1-Palmitoyl-GPC, PAF, and 1-hexadecyl-GPC were obtained from Sigma. L-Asparagine and L-aspartic acid were from Wako (Osaka, Japan).

Enzyme Purification and Partial Amino Acid Sequence—60-kDa lysophospholipase was purified as described previously (25) except that the G-buty1 column step was omitted and 10% glycerol was used instead of dimethyl sulfoxide during S-200 column chromatography. Peptides produced by digestion of the purified enzyme with lysyl endopeptidase were separated by an octylsilane column (2 × 150 mm, Capcell Pak C8, Shiseido) using a 120-min linear gradient of acetonitrile concentration from 0 to 45% (v/v) in 0.05% (v/v) trifluoroacetic acid, at a flow rate of 0.15 ml/min and then sequenced as described previously (29) using the Applied Biosystems model 476A gas phase protein sequence analyzer (Foster City, CA).

Preparation of Antiserum—Two rabbits were anesthetized, injected into popliteal lymph nodes with 30 μg of purified lysophospholipase mixed with 5 μg of killed Mycobacterium butyricum (Difco) and emulsified in complete Freund’s adjuvant (Difco) and then injected intramuscularly with 2 × 1011 killed cells of Bordetella pertussis. Every 4 and 6 weeks, booster injections were performed subcutaneously in the backs of the animals with 50 μg of enzyme emulsified with 5 μg of M. butyricum in complete Freund’s adjuvant. Antiserum was obtained 2 weeks after the final booster injection.

Construction of Rat Liver cDNA Libraries—Total RNA was extracted from male rat liver (1 g) using Isogen (Nippon Gene, Toyama, Japan) according to the manufacturer’s instruction. Poly(A)+ RNA was fractionated from total RNA using Oligotex-dT30 Super (Nippon Roche, Tokyo, Japan). Random-primed and oligo(dT)-primed cDNA libraries were prepared using a cDNA synthesis kit (Amersham Pharmacia Biotech), ligated to the agt11 and agt10 arms (Promega, Madison, WI), respectively, and then packaged using Giga Pack II Gold packaging extracta (Stratagene, La Jolla, CA).

Cloning of Lysophospholipase cDNA and Nucleotide Sequence—The rat liver random-primed agt11 cDNA library was screened with anti-lysophospholipase serum diluted 1:500 as described by Huynh et al. (30) with some modifications. Briefly, plaques from E. coli Y1090r- infected with the recombinant agt11 phages were imprinted on isopyropyl-β-D-thiogalactopyranoside-impregnated nitrocellulose filters (Amersham Pharmacia Biotech), treated with lysophospholipase antiserum and goat anti-rabbit IgG-alanine phosphatase conjugate, and then stained with the ProtoBlot Immunoscreening System (Promega). A positive plaque was amplified in Y1090r-, and its insert was subcloned into plBluescript II SK− (Stratagene). Clone H-1 thus obtained was linearized and used for preparing a radiolabeled RNA probe with the T7 RNA synthesis kit (Nippon gene) and [α-32P]UTP. The probe was hybridized with the oligo(dT)-primed agt10 cDNA library on Hybond-N filters (Amersham Pharmacia Biotech) in a solution containing 48% (v/v) formamide, 4.8 × SSC (0.72 M NaCl in 0.072 M sodium citrate, pH 7.0), 1 × Denhardt’s solution (31), 10% (v/v) dextran sulfate, and 0.1% (w/v) SDS at 42 °C overnight. The filters were washed twice with 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 their inserts were subcloned into plBluescript II SK−. Nucleotide sequences were determined on both strands using a DNA sequencing kit (Perkin Elmer, Foster City, CA) with the 373A DNA sequencer (Perkin-Elmer).

Expression of Lysophospholipase cDNA in HEK293 Cells—In order to obtain the open reading frame, clone I-2, containing the entire open reading frame with the shortest 5′-noncoding sequence, was cleaved with NotI and cloned into pREP9 (Invitrogen, San Diego, CA). The resulting plasmid carrying the insert in the correct orientation (pREP-Ly) was selected, transfected into HEK293 cells by the calcium phosphate precipitation method (32), and cultured in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) containing 5% (v/v) fetal calf serum (Commonwealth Serum Laboratories, Melbourne, Australia). After 44 h, the cells were scraped, harvested by centrifugation at 200 g for 10 min, and then suspended in 0.2 ml of ice-cold lysis buffer (20 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin A, 2 μg/ml antipain, 1 μg/ml chymostatin, 10 μg/ml benzamidophenanthroline, 1 mM benzamidothiobenzothiazol, and 10% (v/v) glycerol). The collected cells were disrupted by sonication for 20 s at an output of 75 watts, centrifuged at 200 × g to remove cell debris, and centrifuged at 10,000 × g for 20 min. The pellet was suspended in 0.2 ml of the lysis buffer and then used for immuno blot analysis and enzyme assays.

Immunoblot Analysis—The proteins were separated by SDS-poly-

RESULTS

Cloning and Sequencing of 60-kDa Lysophospholipase cDNA—Cloning of the complete cDNA for 60-kDa lysophospholipase was carried out as follows. Plaque replicates (1 × 106 plaques) from a rat liver random-primed agt11 cDNA library were screened using two separate batches of antibody raised against the purified rat liver enzyme (25). One positive clone recognized by both batches of antibody was saved, and its insert was cloned into plBluescript II SK−. The resulting plasmid, H-1 (Fig. 1), was used for the preparation of the radiolabeled RNA probe, which was then used for screening an oligo(dT)-primed radiolabeled cDNA library (3 × 106 plaques). Out of the 28 positive clones obtained, two 5′-extended clones were recloned into plBluescript II SK− to obtain clones I-1 and I-2 (Fig. 1). The two sequences were identical except that I-1 was slightly more 5′-extended than I-2. The 2,539-base pair sequence of clone I-1 is shown in Fig. 2. Within the sequence, there was a large open reading frame encoding 564 amino acid residues with a calculated molecular mass of 60,794 Da. We assumed that the ATG at positions 80—82 as the initiation codon for the following reasons. First, the predicted molecular mass was
consistent with the size of the enzyme determined on SDS-polyacrylamide gel electrophoresis. Second, the assumed open reading frame expressed lysophospholipase activity in HEK293 cells (see below). Third, the surrounding sequence, CCAGCCAUUGG, conformed to the consensus sequence for the translational initiation in higher eukaryotes (37). The deduced amino acid sequence contained the partial peptide sequences, SHLV- and Thr89–Asp90–Lys162 in E. coli asparaginase II (52), Thr100–Asp101–Lys173 in Pseudomonas 7A asparaginase (50), and Thr93–Asp94–Lys166 in W. succinogenes asparaginase (51). As shown in Fig. 3B, comparison of the equivalent region of 60-kDa lysophospholipase with those of the three asparaginase suggests that the asparaginase triad of the lysophospholipase is Thr116–Asp117–Lys188.

The remarkable feature of the C-terminal region of 60-kDa lysophospholipase was the 2-fold repeat of 33 amino acid residues (repeat 1, residues 405–437; repeat 2, residues 438–470), which was similar to the ankyrin repeat region of cytoskeleton-associated protein, ankyrin. Thus, the enzyme protein comprised two regions, the asparaginase I-like region and the ankyrin repeat (Fig. 2). Furthermore, there was a leucine zipper motif associated with the asparaginase I-like region (residues 8–361) and residues 109–118 (GFVVIHGTTDT). It is known that the active site of microbial asparaginases is structurally highly conserved and includes three conserved amino acids called the “asparaginase triad,” Thr-Asp-Lys (50, 51). From crystallographic and biochemical studies, the triad was assigned to Thr26–Asp27–Lys162 in E. coli asparaginase II (52), Thr100–Asp101–Lys173 in Pseudomonas 7A asparaginase (50), and Thr93–Asp94–Lys166 in W. succinogenes asparaginase (51).

Expression of 60-kDa Lysophospholipase cDNA in HEK293 Cells—Attempts to express 60-kDa lysophospholipase as a β-galactosidase fusion in E. coli cells were unsuccessful. Therefore, we decided to use mammalian cells as the expression system. cDNA clone I-2 was cleaved with NotI, and the fragment obtained was cloned into the pREP9 expression vector carrying the Rouse sarcoma virus promoter. The resulting plasmid, pREX-Ly, contained nucleotide positions 75–2,538 encoding the entire open reading frame. HEK293 cells were transfected with the plasmid by calcium phosphate precipitation and lysophospholipase assay, since a major protein band recognizable by antiserum against the enzyme. The remarkable feature of the C-terminal region of 60-kDa lysophospholipase was the 2-fold repeat of 33 amino acid residues (repeat 1, residues 405–437; repeat 2, residues 438–470), which was similar to the ankyrin repeat region of cytoskeleton-associated protein, ankyrin. Thus, the enzyme protein comprised two regions, the asparaginase I-like region and the ankyrin repeat (Fig. 2). Furthermore, there was a leucine zipper motif associated with the asparaginase I-like region (residues 8–361) and residues 109–118 (GFVVIHGTTDT). It is known that the active site of microbial asparaginases is structurally highly conserved and includes three conserved amino acids called the “asparaginase triad,” Thr-Asp-Lys (50, 51). From crystallographic and biochemical studies, the triad was assigned to Thr26–Asp27–Lys162 in E. coli asparaginase II (52), Thr100–Asp101–Lys173 in Pseudomonas 7A asparaginase (50), and Thr93–Asp94–Lys166 in W. succinogenes asparaginase (51). As shown in Fig. 3B, comparison of the equivalent region of 60-kDa lysophospholipase with those of the three asparaginase suggests that the asparaginase triad of the lysophospholipase is Thr116–Asp117–Lys188.

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Since the enzyme bears significant sequence similarity to microbial asparaginases, we were interested to examine whether or not the transformant contained asparaginase activity. The homogenate of the transformant was incubated with [G-3H]asparagine, and the aspartic acid formed was separated by DEAE-cellulose paper chromatography and counted. As shown in Fig. 4C, the asparaginase activity of the transformant was 4.3 times as high as that of the vector control, indicating that the expressed enzyme indeed exhibited asparaginase activity. Aarsman et al. (28) previously showed that the 60-kDa
lysophospholipase from bovine liver (lysophospholipase II according to their nomenclature (11)) contained PAF acetylhydro-lase activity. Thus, we were interested in examining whether or not the expressed enzyme exhibited PAF acetylhydrolase activity. As shown in Fig. 4 D, the transformant exhibited PAF acetylhydrolase activity. The activity was 2.1 times higher than that of the vector control, confirming that the enzyme contains PAF acetylhydrolase activity as an intrinsic activity. It is interesting to note that calcium-independent phospholipase A2, which seemingly resembled the present enzyme in carrying the ankyrin repeat, also exhibits PAF acetylhydrolase (6). The increases in the activities of lysophospholipase (2.5-fold), asparginase (4.3-fold), and PAF acetylhydrolase (2.1-fold) by transformation appeared to be somewhat lower than expected from the immunoblot data (Fig. 4 A), but this was partly due to the endogenous activities present in HEK293 cells. A possibility also exists that the enzyme required an as yet unidentified activator protein, deficient in HEK293 cells (see “Discussion”). In agreement with the above results, the purified enzyme showed asparaginase and PAF acetylhydrolase activities with the relative hydrolytic rates of 100:38:32, the data confirming that the 60-kDa lysophospholipase contains asparaginase and PAF acetylhydrolase activities.

Tissue Distribution of 60-kDa Lysophospholipase mRNA—To determine the size of 60-kDa lysophospholipase mRNA, we isolated poly(A)+ RNA from rat liver and performed Northern blot analysis using the 32P-labeled Bal I fragment of clone I-1 (nucleotide positions 116–573) as the probe. As shown in Fig. 5 A, a 2.3-kilobase band hybridized to the probe under the high stringency condition. Thus, the size of the transcript was fairly consistent with that of the cloned cDNA. Although clearly observed in liver, the transcript was not detectable in heart and lung (data not shown), suggesting that the transcript was expressed in a tissue-specific manner. Thus, we examined the tissue distribution of 60-kDa lysophospholipase mRNA by RT-PCR using a pair of specific primers (Fig. 2). As shown in Fig. 5 B, 60-kDa lysophospholipase mRNA was abundantly expressed in liver and kidney and at a low level in stomach, but it was hardly seen in spleen, lung, heart, and brain. Thymus showed a weak, slowly moving band, whose entity was unknown. The results obtained here indicate that 60-kDa lysophospholipase mRNA is distributed in limited tissues.

DISCUSSION

This paper reports the initial cloning of large form lysophospholipase in mammalian tissues. The sequence bears little sequence similarity to that of small form lysophospholipase (12, 20). Small form lysophospholipase resembles the esterases of Pseudomonas fluorescens (21) and Spirulina platensis (22) and contains the esterase/lipase consensus, Gly-X-Ser-X-Gly (12, 20). The serine in the consensus is part of the esterase catalytic triad, Ser-Asp-His, and plays a role of nucleophile in the hydrolytic reaction of the enzyme. In P388 macrophage lysophospholipase, the catalytic triad was assigned to Ser119–Asp174–His208 (67). In contrast to the small form lysophospholipase, large form lysophospholipase resembles microbial asparaginase. Indeed, the large form lysophospholipase cloned here catalyzed the hydrolysis of asparagine. The mechanism of the asparaginase reaction is reminiscent of that of serine esterase. However, threonine, rather than serine, has been postulated to play a role of nucleophile (52), and lysine, instead of histidine, is thought to enhance the nucleophilicity of the threonine (51). Crystallographic and biochemical studies identified the asparaginase catalytic triad as Thr89-Asp90–Lys162 for E. coli asparaginase II (52), Thr100–Asp101–Lys173 for Pseudomonas 7A...
asparaginase (50), and Thr93-Asp94-Lys166 for W. succinogenes asparaginase (51). Sequence alignment of 60-kDa lysophospholipase with these bacterial asparaginases suggests that the catalytic triad of the enzyme might be Thr116-Asp117-Lys188, which had well conserved surrounding sequences. It is tempting to speculate that these amino acids play an essential role in the catalytic activity of 60-kDa lysophospholipase and that the Thr116 is the nucleophile involved in the formation of the acyl-enzyme intermediate.

From the results of RT-PCR, mRNA encoding 60-kDa lysophospholipase is expressed at high levels in liver and kidney and a low level in stomach but at negligible levels in other tissues. Lysophospholipase-transacylase partially purified from gastric mucosa (27) showed similar properties to the rat liver enzyme. It had a pH optimum at 6.0 with the \( K_m \) value of 0.25 mM for lyso-PC, but it was only 10% active at pH 8.5. The isoelectric point was determined to be 5.4, while the value for

**FIG. 3.** Comparison of 60-kDa lysophospholipase with E. coli asparaginase I (A), bacterial asparaginases (B), and various ankyrin repeats (C). The \( n \)-numbers indicate the first and last amino acid positions. **Boldface letters** indicate identical amino acids (A and B) or those conforming to the ankyrin repeat consensus (53) (C). In panel A, the sequence of 60-kDa lysophospholipase was compared with that of E. coli asparaginase I using the MACAW program (70). Lyso, lysophospholipase; AspI, E. coli asparaginase I. In panel B, the sequence of 60-kDa lysophospholipase is aligned to those of the bacterial asparaginases whose catalytic triads had been identified as indicated by the arrowheads. Alignment was made using the multiple alignment program (71). Lyso, lysophospholipase; EcoII, E. coli asparaginase II; Pse, Pseudomonas 7A asparaginase; Wol, W. succinogenes asparaginase. In panel C, each of the two ankyrin repeat units (repeat 1, 405–437; repeat 2, 438–470) of 60-kDa lysophospholipase was compared with the protein data bases using the BLAST algorithm (39), and the highest scoring sequences were aligned to the ankyrin repeat consensus (53).

**FIG. 4.** Expression of lysophospholipase cDNA in HEK293 cells. A, immunoblot analysis. 100 ng of the purified enzyme (lane 1) and 20 μg of extracts from HEK293 cells transformed with pREP9 (lane 2) or pREX-Ly (lane 3) were subjected to SDS-PAGE, followed by immunoblot analysis using anti-lysophospholipase serum as described under “Experimental Procedures.” The arrows indicate the locations of molecular mass markers: bovine serum albumin, 84 kDa; carbonic anhydrase, 44.3 kDa; soybean trypsin inhibitor, 32.8 kDa. B, lysophospholipase activity. C, asparaginase activity. D, PAF acetylhydrolase activity. HEK293 cells were transformed with pREP9 (lane 1) or pREX-Ly (lane 2) and cultured for 44 h. 90 μg of cell extracts were assayed for lyso-PC-hydrolyzing activity (B), asparaginase activity (C), or PAF acetylhydrolase activity (D) as described under “Experimental Procedures.”

**The star** in the cactus sequence denotes dipeptide, Gly-Lys. Lyso, lysophospholipase; CDK6I, human cyclin-dependent kinase 6 inhibitor; CDK4I, human cyclin-dependent kinase 4 inhibitor D; rSMPP, rat smooth muscle protein phosphatase 1M (chain M110); sSMPP, chicken smooth muscle protein phosphatase (large chain, long form); IeBRP, human IeB-related protein; cactus, D. melanogaster ankyrin repeat acidic protein cactus; notch3, mouse notch 3 protein; DGK, eye-specific diacylglycerol kinase; ANK3, human long form ankyrin 3; ANK2, human brain ankyrin 2; p53BP, human p53-binding protein 2; ANK1, human erythrocyte ankyrin 1; DAPK, human death-associated protein kinase; res2, S. pombe cell cycle regulator; NRF2, human nuclear respiratory factor 2 (β2-chain).
lysophospholipase II catalyzed the transacylase activity with Triton X-100 and purified it by a somes. Furthermore, we could solubilize lysophospholipase-zyme. The antibody recognized a 60-kDa protein in micro-
liver microsomes using the antibody against the 60-kDa en-
verse. The results are consistent with the view that a different isoform of
such as heart (24) and lung (26), clearly showing that heart and
involves in enzyme activity through oligomeric complex forma-
exists in a complexed form with some other protein that may
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REFERENCES
1. Fujimori, Y., Kudo, I., Fujita, K., and Inoue, K. (1993) Eur. J. Biochem. 218, 629–635.
2. Reynolds, L. J., Hughes, L. L., Louis, A. I., Kramer, R. M., and Dennis, E. A. (1993) Biochem. Biophys. Acta. 1167, 272–280.
3. de Carvalho, M. S., McCormack, F. X., and Leslie, C. C. (1993) J. Biol. Chem. 268, 229–238.
4. Pete, M. J., and Exton, J. H. (1996) J. Biol. Chem. 271, 1302–1309.
5. Ackerman, S. J., Corrette, S. E., Rosenberg, H. F., Bennett, J. C., Mastrianni,
6. Tang, J., Kriz, R. W., Wolfman, N., Shaffer, M., Seehra, J., and Jones, S. S. (1997) J. Biol. Chem. 272, 8567–8575.
7. de Jong, J. G., van den Bosch, H., Rijken, D., and van Deenen, L. L. (1974) Biochim. Biophys. Acta. 369, 50–63.
8. de Jong, J. G., van den Bosch, H., Rijken, D., and van Deenen, L. L. (1974) Biochim. Biophys. Acta. 369, 50–63.
9. Waite, M. (1991) in Biochemistry of lipids. Lipoproteins and membranes (Vance, D. E., and Vance, J. eds) pp. 269–289, Elsevier Science Publishers B. V., Amsterdam.
10. de Jong, J. G., van den Bosch, H., Rijken, D., and van Deenen, L. L. (1974) Biochim. Biophys. Acta. 369, 50–63.
11. Gross, R. W., and Sobel, B. E. (1983) J. Biol. Chem. 258, 2583–2588.
12. Gross, R. W., and Sobel, B. E. (1983) J. Biol. Chem. 258, 2583–2588.
13. Gross, R. W., and Sobel, B. E. (1983) J. Biol. Chem. 258, 2583–2588.
14. Gross, R. W., and Sobel, B. E. (1983) J. Biol. Chem. 258, 2583–2588.
15. Zhang, Y. Y., and Dennis, E. A. (1988) Biochim. Biophys. Acta 956, 299–309.
16. Waite, M. (1991) in Biochemistry of lipids. Lipoproteins and membranes (Vance, D. E., and Vance, J. eds) pp. 269–289, Elsevier Science Publishers B. V., Amsterdam.
17. Garsetti, D. E., Steiner, M. R., Holtsberg, F., Bennett, J. C., Mastrianni,
18. Garsetti, D. E., Steiner, M. R., Holtsberg, F., Bennett, J. C., Mastrianni,
19. Garsetti, D. E., Steiner, M. R., Holtsberg, F., Bennett, J. C., Mastrianni,
20. Wang, A., Deems, R. A., and Dennis, E. A. (1997) J. Biol. Chem. 272, 1243–1250.
21. Hong, K. H., Jang, W. H., Choi, K. D., and Yoo, O. J. (1991) Agric. Biol. Chem. 55, 2389–2395.
22. Salvi, S., Trinei, M., Lanfalon, F., and Pon, C. L. (1994) Mol. Genet. Genomics 243, 124–126.
23. Ackerman, S. J., Corrette, S. E., Rosenzweig, H. F., Bennett, J. C., Mastrianni,
24. Ackerman, S. J., Corrette, S. E., Rosenzweig, H. F., Bennett, J. C., Mastrianni,
25. Ackerman, S. J., Corrette, S. E., Rosenzweig, H. F., Bennett, J. C., Mastrianni,
26. Brumley, G., and van den Bosch, H. (1977) Eur. J. Biochem. 213, 215–220.
27. Ackerman, S. J., Corrette, S. E., Rosenzweig, H. F., Bennett, J. C., Mastrianni,
28. Ackerman, S. J., Corrette, S. E., Rosenzweig, H. F., Bennett, J. C., Mastrianni,
29. Ackerman, S. J., Corrette, S. E., Rosenzweig, H. F., Bennett, J. C., Mastrianni,
30. Ackerman, S. J., Corrette, S. E., Rosenzweig, H. F., Bennett, J. C., Mastrianni,
Lysophospholipase cDNA

46. Minton, N. P., Bullman, H. M., Scawen, M. D., Atkinson, T., and Gilbert, H. J. (1986) Gene (Amst.) 46, 25–35.
47. Tanaka, S., Robinson, E. A., Appella, E., Miller, M., Ammon, H. L., Roberts, J., Weber, I. T., and Wlodawer, A. (1988) J. Biol. Chem. 263, 8583–8591.
48. van Dijl, J. M., de Jong, A., Smith, H., Bron, S., and Venema, G. (1991) FEMS Microbiol. Lett. 65, 349–351.
49. Sun, D. X., and Setlow, P. (1991) Proc. Natl. Acad. Sci. U. S. A. 87, 1730–1734.
50. Guan, K. L., Jenkins, C. W., Li, Y., Nichols, M. A., Wu, X., O’Keefe, C. L., Matera, A. G., and Xiong, Y. (1994) Genes Dev. 8, 2939–2952.
51. Haystead, T. A., Hui, H., Bowman, J., Zeldin, D., and Lassman, E. B. (1996) FEBS Lett. 381, 123–127.
52. Wang, A., Loo, R., Chen, Z., and Dennis, E. A. (1997) J. Biol. Chem. 272, 22030–22036.
53. van den Bosch, H., and de Jong, J. G. (1975) Biochim. Biophys. Acta. 398, 244–257.
54. Lux, S. E., John, K. M., and Bennett, V. (1990) Nature 344, 36–42.
55. Schuler, G. D., Altschul, S. F., and Lipman, D. J. (1991) Proteins 9, 180–190.
56. Barton, G. J., and Sternberg, M. J. (1987) J. Mol. Biol. 196, 327–337.