Recently, a novel enzyme, 1-O-acylceramide synthase (ACS), was purified and characterized from bovine brain. This enzyme has both calcium-independent phospholipase A₂ and transacylase activities. The discovery of this enzyme led us to propose a new pathway for ceramide metabolism in which the sn-2-acyl group of either phosphatidylethanolamine or phosphatidylcholine is transferred to the 1-hydroxy group of ceramide. In this study, the partial amino acid sequences from the purified enzyme revealed that the enzyme contains amino acid sequences identical to those of human lecithin:cholesterol acyltransferase-like lysophospholipase (LLPL). The coding sequences of the mouse, bovine, and human genes were obtained from the respective kidney cDNAs by PCR. The open reading frames of LLPL were cloned into pcDNA3 to generate carboxyl-terminally tagged proteins. The expression of mouse LLPL in COS-7 cells demonstrated that transfected cells had higher transacylase and phospholipase A₂ activities than did non-transfected cells. Immunoprecipitation confirmed that LLPL had ACS activity. There were no significant lecithin:cholesterol acyltransferase and lysophospholipase activities in the mouse LLPL-transfected cells under either acidic or neutral conditions. Amino acid sequences from cDNAs of mouse, human, and bovine LLPLs demonstrated a signal peptide cleavage site, one lipase motif (AXSXG), and several N-linked glycosylation sites in each LLPL molecule. The replacement of serine with alanine in the lipase motif of mouse LLPL resulted in elimination of enzyme activity, indicating that the serine residue is part of the catalytic site. Deglycosylation of mouse, human, and bovine LLPLs yielded core proteins with a molecular mass of 42 kDa without change in enzyme activities. LLPL was post-translationally modified by signal peptide cleavage and N-linked glycosylation, and each mature LLPL had the same size core protein. Subcellular fractionation demonstrated that ACS activity co-localized with N-acetylgalactosaminidase. Therefore, LLPL encodes a novel lysosomal enzyme, ACS.

For the last decade, ceramide has been thought to play an important role in cell signal transduction involving cell growth, proliferation, differentiation, stress responses, and apoptosis (1). The ceramide levels within cells are regulated by several well defined metabolic pathways. We recently studied the metabolism of N-acetylsphingosine (NAS) in Madin-Darby canine kidney (MDCK) cells (2). In that study, NAS was actively metabolized and was not an inert compound, as had been previously suggested (3). NAS was converted to other sphingolipids, including sphingosine, C₂-sphingomyelin, C₂-glucosylceramide, long-chain ceramide, long-chain sphingomyelin, and long-chain glucosylceramide. An unexpected product was also detected. This metabolite was a highly nonpolar compound and identified as 1-O-acyl-NAS.

This discovery led to the discovery of a new enzyme activity, one that catalyzes the esterification of the hydroxyl group at C-1 in the ceramide molecule under acidic conditions. The enzyme does not require divalent cations for its activity. Glycosphingolipids (in particular, phosphatidylethanolamine (PE) and phosphatidylcholine (PC)) were identified as acyl group donors in the reaction. The acyl group at the sn-2-position in the phospholipid is transferred to an acceptor molecule, e.g. ceramide or water. If the acceptor is ceramide, 1-O-acylceramide is formed. However, if the acceptor is water, free fatty acid is released. It was also observed that a short-chain rather than a long-chain ceramide is preferred as an acceptor. These observations raised the possibility that this new enzyme regulates a novel pathway of ceramide metabolism.

The new enzyme, named 1-O-acylceramide synthase (ACS), was purified from bovine brain and further characterized (4). ACS is a water-soluble glycoprotein with a molecular mass of 45 kDa and a single polypeptide chain, which specifically binds to concanavalin A-conjugated agarose. The enzyme has a pH optimum at 4.5 and has both phospholipase A₂ and transacylase activities. The enzyme activity is calcium-independent. Therefore, ACS may be classified as a calcium-independent phospholipase A₂.

In this study, we report that a BLAST search revealed that the peptides obtained from ACS purified from bovine brain share amino acid sequences with a recently reported human gene termed lecithin:cholesterol acyltransferase (LCAT)-like lysophospholipase (LLPL) (5). To understand the biological function of ACS, the ACS gene was sequenced on the basis of its similarity to LLPL, and the gene products were characterized.

MATERIALS AND METHODS

Reagents—The reagents and sources were as follows: hemagglutinin (HA) and c-Myc peptides and mouse anti-HA monoclonal antibody.

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(clone 12C5A5) from Roche Molecular Biochemicals; anti-c-Myc monoclonal antibody (clone 9E10, mouse ascites fluid), anti-FLAG monoclonal antibody M2, horseradish peroxidase-conjugated goat anti-mouse IgG antibody, diaminobenzidine, PE from bovine brain, diethyl phosphate, and CAPS from Sigma; PVDF membrane (Westran) from Schleicher & Schuell. Bovine serum albumin (Fraction V) and dioleoylphosphatidylcholine from Avanti.

Determination of the Amino-terminal Amino Acid Sequence and Partial Amino Acid Sequences of Tryptic Fragments of Bovine Brain ACS—ACS was purified from bovine brain as previously described (4). The protein content was determined by the bicinchoninic acid protein assay (Pierce) with bovine serum albumin as a standard. SDS-PAGE was performed following the method of Laemmli and Favre (6).

For determination of the amino-terminal amino acid sequence of ACS, the purified protein was separated using a 10% acrylamide gel with bovine serum albumin as a standard. SDS-PAGE was performed after the method of Laemmli and Favre (6).

For determination of the amino-terminal amino acid sequence of ACS, the purified protein was separated using a 10% acrylamide gel and transferred to a PVDF membrane using transfer buffer (10 mM ACS, the purified protein was separated using a 10% acrylamide gel (Pierce) with bovine serum albumin as a standard. SDS-PAGE was performed following the method of Laemmli and Favre (6).

For determination of the amino-terminal amino acid sequence of ACS, the purified protein was separated using a 10% acrylamide gel and transferred to a PVDF membrane using transfer buffer (10 mM CAPS (pH 11) in 10% methanol) at a constant voltage of 50 V for 60 min at room temperature. The protein on the membrane was briefly stained with 0.1% Coomassie Brilliant Blue R-250 in 40% methanol and 1% acetic acid and destained with 50% methanol. The membrane was extensively rinsed with deionized water, and the protein band was excised. The excised PVDF sample was air-dried and analyzed in the Howard Hughes Medical Institute Biopolymer/W. M. Keck Foundation Biotechnology Research Laboratory at Yale University.

For determination of partial amino acid sequences of tryptic fragments of ACS, the protein band in the gel was stained with 0.1% Coomassie Brilliant Blue R-250 in ethanol/acetic acid/water (9:2:9), extensively destained with ethanol/acetic acid/water (25:8:65), and excised. The gel slice was washed twice with 50% acetonitrile. Tryptic digestion of the band and sequence analysis were carried out at the Harvard Microchemistry Facility by microcapillary reverse-phase high performance liquid chromatography nanoelectrospray tandem mass spectrometry on a Finnigan LCQ DECA quadrupole ion trap mass spectrometer.

**Cloning of Mouse and Bovine LLPLs—**Total RNAs were extracted from mouse and bovine kidneys with Trizol (Invitrogen). Reverse transcription was performed according to the directions included with the SuperScript™ system (Invitrogen). Human kidney cDNA was purchased from Research Genetics. The PCR amplifications employed 35 cycles with steps at 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1.5 min with platinum Pfx DNA polymerase (Invitrogen) and kidney cDNAs as templates. The primers used for PCR were 5'-CCCAATGGATGAGTCCGACCATCTC-3' and 5'-CCGGGCTAGGTCAGAAGCACACGTTT-3' for mouse LLPL, 5'-CCAACTTGGATGTTCTGCTCTG-3' and 5'-CCGGTCTGAGGCGGGCTAAGAAGCACAGACCTT-3' for bovine LLPL, 5'-CCACCTTGGATGTTCTGCTCTG-3' and 5'-CCGGTCTGAGGCGGGCTAAGAAGCACAGACCTT-3' for human LLPL. PCR product ligates were ligated into the pcRI-TOPO vector (Invitrogen), followed by transformation into E. coli DH5α (Invitrogen).

**Constitution of LLPL Expression Plasmids—**The entire open reading frames of the individual LLPLs were excised at HindIII and XhoI sites from the plasmids described above. They were then subcloned into the HindIII and XhoI sites of pcDNA3-FLAG, pcDNA3-HA, or pcDNA3-c-Myc (all three generously provided by Dr. Naohiro Inohara) to generate carboxyl-terminally tagged LLPL proteins. The mutation of serine to alanine in the putative lipase motif sequence of mouse LLPL was generated by the overlap extension method using primers Mt1-1F (5'-GCCCAACGCTTGGGCGACAC-3') and Mt1-1R (5'-TTGGCCCAT-AGCCTGGGCGACCA-3') (8).

**Cell Culture and Transfection—**COS-7 cells were grown in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal bovine serum. For transient expression, COS-7 cells were cultured in 35-mm dishes. When the cells reached 80% confluence, they were transfected with 1 µg/ml purified plasmid using LipofectAMINE Plus™ (Invitrogen) in 1 ml of Opti-MEM medium (Invitrogen). 1 ml of Dulbecco's modified Eagle's medium containing 20% fetal bovine serum was added after 3 h of incubation at 37 °C and 5% CO2 for 24 h after transfection, the cells were washed three times with 2 ml of phosphate-buffered saline and then reseeded into another 60-mm dish with 5 ml of DMEM. The medium was replaced with 2 ml of DMEM for 20 min at 37 °C and transferred into an ultracentrifuge tube. The following procedures were carried out at 4 °C. The cells were collected by centrifuge at 800 g × 10 min. The pellet of the cells was dispersed into 0.5 ml of 0.25% sucrose and 10 ml HEPES (pH 7.4) by sonication. Sonication was carried out for 4 × 10 s at 0 °C in a probe-type sonicator. The suspension was centrifuged for 1 h at 100,000 × g. The resultant supernatant was collected as a soluble fraction and used in protein analysis and enzyme assay.

**Enzyme Assay (Transacylase Activity)—**The assay conditions are described in the figure legends. In general, liposomes consisting of dioleoylphosphatidylcholine (60.5 mol %), PE (27.3 mol %) and diethyl phosphate (12.2 mol %) in a dry film were hydrated in 50 mM NaPi buffer (pH 7.4) and then treated for 15 h with 100 µM leupeptin and 100 µM pepstatin A. After treatment, the cells were washed twice with 2 ml of cold phosphate-buffered saline, scraped with 3 ml of cold 0.25% sucrose and 1 ml of 100 mM EDTA (pH 7.4) (buffer A) and transferred into a 15-ml plastic tube. The following procedures were subsequently carried out at 4 °C. Another 3 ml of buffer A was added to recover the remaining cells, and

**Preparation of Lysosomes—**The lysosome preparation was performed using the method of Rohrer et al. (10) with slight modifications. Confluent MDCK cells in a 15-cm dish were cultured with 21 ml of Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum and then treated for 15 h with 100 µM leupeptin and 100 µM pepstatin A. After treatment, the cells were washed twice with 21 ml of cold phosphate-buffered saline, scraped with 3 ml of cold 0.25% sucrose and 1 ml of 100 mM EDTA (pH 7.4) (buffer A), and transferred into a 15-ml plastic tube. The following procedures were subsequently carried out at 4 °C. Another 3 ml of buffer A was added to recover the remaining cells, and
A Lysosomal Phospholipase A₂

The cells were collected by centrifugation at 200 × g for 10 min and suspended with 2.5 ml of buffer A. The cell suspension was homogenized with 20 strokes of a ground-glass pestle homogenizer. The homogenate was diluted 2-fold with buffer A and centrifuged for 10 min at 400 × g. The post-nuclear supernatant was centrifuged at 12,000 × g for 20 min. The pellet obtained from the post-nuclear supernatant was resuspended in 1 ml of buffer A and then diluted with 1 ml of buffer A.

**Results**

cDNA Cloning of the LLPL Gene—ACS was purified from bovine brain, and the tryptic fragments were sequenced by mass spectrometry. A BLAST search revealed that the partial amino acid sequences of ACS are highly homologous to the amino acid sequences of human, mouse, and bovine LLPLs. Therefore, the gene product expressed from ACS was presumed to be identical to ACS.

Expression of Mouse LLPL in COS-7 Cells—The mouse LLPL gene was used in the first expression study of LLPL. The entire open reading frame of LLPL was cloned into the HindIII and XhoI sites of pcDNA3 to generate carboxyl-terminally tagged proteins with FLAG, HA, or c-Myc peptides. Each vector was transfected into COS-7 cells with LipofectAMINE. ACS activity in the soluble fraction of the cell homogenate was observed using liposomes consisting of PC, PE, dicetylphosphate, and NAS under acidic conditions. The soluble fraction prepared from mouse LLPL-transfected cells catalyzed marked formation of 1-O-acyl-NAS and release of fatty acid as a function of incubation time (Fig. 3). Also, a similar enzyme activity was observed in each soluble fraction from cells transfected with carboxyl-terminally FLAG-, HA-, and c-Myc-tagged LLPLs. The soluble fraction from each mouse LLPL-transfected cell preparation had ~30-fold increased activity compared with the non-transfected cells. This indicates that the LLPL gene product expressed from ACS is identical to ACS.
transacylase activity compared with that from non-transfected cells (Fig. 3). The carboxyl-terminal tags did not affect LLPL activity or expression. These results indicate that recombinant LLPL has the same transacylase and phospholipase A2 activities as ACS.

**Confirmation of ACS Activity of LLPL Expressed in LLPL-transfected Cells**—Immunoprecipitations were employed to demonstrate that recombinant LLPL itself has ACS activity. The soluble fraction of c-Myc-tagged mouse LLPL-transfected COS-7 cells was incubated with anti-c-Myc monoclonal antibody and protein G-agarose beads. In this assay, the resultant protein G-agarose beads were incubated with liposomes containing NAS. When the soluble fraction was incubated with anti-c-Myc antibody, formation of 1-O-acyl-NAS and release of fatty acid were observed with protein G-agarose beads (Fig. 4, lane 3). However, when the soluble fraction was incubated with anti-c-Myc antibody in the presence of an excess of c-Myc peptide, no significant products were found with protein G-agarose beads (Fig. 4, lane 4). On the other hand, when the soluble fraction was incubated with anti-c-Myc antibody in the presence of an excess of HA peptide, formation of 1-O-acyl-NAS and release of fatty acid were observed with protein G-agarose beads (Fig. 4, lane 5). These results demonstrate that the recombinant protein expressed in cells transfected with c-Myc-tagged mouse LLPL specifically binds to anti-c-Myc monoclonal antibody and shows dual transacylase and phospholipase A2 activities at pH 4.5. This experiment confirmed that LLPL expressed in cells transfected with cDNA encoding LLPL has ACS activity. Therefore, ACS is a gene product of the LLPL gene.

**LCAT and Lysophospholipase Activities of Mouse LLPL**—
According to Taniyama et al. (5), the deduced amino acid sequence of human LLPL is 49% homologous to human LCAT, although human LLPL did not display LCAT activity under their neutral assay conditions. We determined whether mouse recombinant LLPL has LCAT activity. As expected from the previous results (Fig. 4), a marked phospholipase A2 activity was observed in the soluble fraction from LLPL-transfected cells under acidic conditions (Fig. 5). However, no significant esterification of cholesterol was observed in the same soluble fractions under either acidic or neutral conditions (Fig. 5).

Taniyama et al. (5) also reported that human recombinant LLPL has lysophospholipase activity. We investigated lysophospholipase activity in the soluble fraction from LLPL-transfected cells. Interestingly, the soluble fraction did not show any significant increase in lysophospholipase activity under either acidic or neutral conditions (Fig. 5). However, no significant esterification of cholesterol was observed in the same soluble fractions under either acidic or neutral conditions (Fig. 5).

Point Mutation of a Putative Lipase Motif in LLPL—LLPL has a putative lipase motif, $A_XS_XG$ (Fig. 1). A serine-to-alanine substitution in the $A_XS_XG$ sequence of LLPL was generated by the overlap extension method using PCR (8). The soluble fraction of the mutated LLPL-transfected cells did not have any significant enzyme activity (Fig. 7A). Western blot analysis showed that the protein expression of mutated LLPL in COS-7 cells was comparable to that of LLPL (Fig. 7B). These results strongly support the conclusion that the serine residue in the lipase motif is essential for enzyme activity.

Post-translational Modification of LLPL—There is a disparity between the apparent molecular mass reported for human LLPL (57 kDa) expressed in COS-7 cells and that observed for purified bovine ACS (45 kDa) upon SDS-PAGE. To resolve this discrepancy, COS-7 cells were transfected with HA-tagged
mouse, human, and bovine recombinant LLPL DNAs, and the expressed LLPLs were studied.

The soluble fraction of the transfected cells had comparable levels of ACS activity (Fig. 7A). The protein expression of mouse, human, and bovine LLPLs was also comparable as confirmed by Western blotting with anti-HA monoclonal antibody (Fig. 8B). HA-tagged mouse, human, and bovine LLPLs expressed in COS-7 cells have molecular masses of 51, 50, and 47 kDa, respectively (Fig. 8B). According to their deduced amino acid sequences, both mouse and human LLPLs have four putative N-linked glycosylation sites. Bovine LLPL has three putative N-linked glycosylation sites. Therefore, the differences in their molecular masses were thought to be due to differences in glycosylation in LLPLs.

Bovine brain ACS binds to concanavalin A-agarose beads and is specifically released with methyl-a-D-mannopyranoside from the beads (4). The treatment of each soluble fraction with endoglycosidase F, an enzyme that cleaves asparagine-linked oligomannose and hybrid oligosaccharides, resulted in a decrease in molecular mass for each LLPL. Bovine LLPL has three putative N-linked glycosylation sites. Therefore, the differences in their molecular masses were thought to be due to differences in glycosylation in LLPLs.

Localization of ACS in Cells—Previous studies showed that ACS has an acidic pH optimum and N-linked oligomannose (2, 4). These properties indicate that ACS is a probably a lysosomal enzyme. A preliminary study showed that ACS activity is relatively high in MDCK cells compared with other animal tissues (data not shown). Therefore, MDCK cells were used to determine the subcellular localization of ACS using the method of Percoll gradient fractionation (10).

11 and 89% of the b-hexosaminidase activity in the post-nuclear supernatant was recovered in the post-mitochondrial supernatant and crude mitochondrial fractions, respectively, after centrifugation at 12,000 × g. Also, 17 and 83% of the ACS activity in the post-nuclear supernatant was recovered in the
post-mitochondrial supernatant and crude mitochondrial frac-
tions, respectively. Cytochrome c oxidase activity was not de-
tected in the post-mitochondrial supernatant. The crude mito-
chondrial fraction was applied to Percoll gradient fractionation
(Fig. 9). 75 and 14% of the β-hexosaminidase activity was
recovered in fractions 1–3 and 9–11, respectively, indicating
that fractions 1–3 represent the lysosome-enriched fraction. 54
and 29% of the ACS activity was recovered in fractions 1–3 and
9–11, respectively.

The difference in the observed β-hexosaminidase and ACS
activities is likely due to the inhibition of ACS by Percoll. 40% of
the transacylase activity in the crude mitochondrial fraction
was inhibited by 0.72% Percoll. In addition, the lipid extract
obtained from the reaction mixture containing fraction 1, 2, or
3, but not fraction 10 or 11, yielded a large Percoll spot at the
TLC plate origin after development in a solvent system con-
sisting of chloroform/acetic acid (9:1). These findings indicate
that ACS activity in fractions 1–3 is likely lowered by the
presence of Percoll, and the actual profile of ACS activity is
similar to that of β-hexosaminidase activity. Furthermore, the
cytochrome c oxidase activity in fraction 10 was ~4-fold higher
than that in fraction 1, indicating that most of the mitochon-
dria were recovered in fractions 9–11. These results support
the conclusion that ACS is a lysosomal enzyme.

**Fig. 7. Expression of mouse LLPL and mutated LLPL in COS-7 cells.** COS-7 cells were transiently transfected with pcDNA3,
pcDNA3-c-Myc, pcDNA3-c-Myc-tagged mouse LLPL, or pcDNA3-c-Myc-tagged mutated mouse LLPL. Each soluble fraction obtained from
non-transfected or transfected cells was used for ACS activity assay (A) and Western blotting (B). In A, 2 μg of each soluble fraction obtained from
non-transfected or transfected cells was incubated for 10 and 20 min at 37 °C with liposomes containing NAS as described under “Materials and
Methods.” In B, 20 μg of protein in each soluble fraction was separated using a 10% acrylamide gel and transferred to a PVDF membrane as
described under “Materials and Methods.” Half of the membrane was blocked with skim milk and then incubated with anti-c-Myc monoclonal
antibody. The antigen-antibody complex on the membrane was visualized with horseradish peroxidase-conjugated goat anti-mouse IgG antibody
using diaminobenzidine and hydrogen peroxide. The rest of membrane was stained with Coomassie Brilliant Blue R-250 (CBB). Cont., control
non-transfected cells; pcDNA, pcDNA3-transfected cells; pcDNA-myc, pcDNA3-c-Myc-transfected cells; LLPL-myc and mLLPL-myc, c-Myc-tagged
mouse LLPL-transfected cells; LLPL-myc(S to A) and mLLPL-myc(S to A), c-Myc-tagged mouse mutated LLPL-transfected cells.
DISCUSSION

This cloning study revealed that the previously reported LLPL gene encodes ACS. The gene product expressed in LLPL-transfected cells has both transacylase and phospholipase A2 activities under acidic conditions, but lacks significant LCAT and lysophospholipase activities under either acidic or neutral conditions.

Previously, Taniyama et al. (5) reported that human LLPL expressed in COS-7 cells is a secreted protein that has lyso-phospholipase activity under neutral conditions. In this study, we failed to observe any significant increase in lysophospholipase activity in the cultured medium of COS-7 cells transfected with recombinant LLPL DNA. This was true even when lysophospholipase activity was assayed at concentrations exceeding the critical micellar concentration of lyso-PC. A very slight but significant increase in lysophospholipase activity in the soluble fraction of the LLPL-transfected cells was observed when assayed at lyso-PC concentrations greater than the critical micellar concentration. The specific activity of lysophospholipase was significantly lower than that of ACS. Purified ACS from bovine brain has weak enzyme activity as a phospholipase A1 (4). Therefore, the lysophospholipase activity observed in recombinant LLPL probably reflects the minor phospholipase A2 activity of ACS. We conclude that the LLPL gene product mainly functions as ACS or as a phospholipase A2, but not as a lysophospholipase.

Human recombinant LLPL is inactivated with diisopropyl fluorophosphate, indicating that the active site contains a serine residue (5). In our study, the replacement of serine with alanine within the lipase motif (A\_X\_S\_X\_G) of recombinant LLPL resulted in a loss of the phospholipase A2 and transacylase activities. Therefore, the serine residue in the motif must be essential for enzyme activity. As reported for human LLPL (5, 14), the catalytic triad of serine 181, aspartic acid 345, and histidine 377 of LCAT is also conserved in mouse and bovine LLPLs (Fig. 1). This observation is consistent with the view that the serine residue in the motif is an active site and that the enzyme forms an acyl-enzyme intermediate via the hydroxyl group of the serine (Fig. 10).

The deduced amino acid sequences of mouse, human, and bovine LLPLs indicate that each entire sequence has a signal sequence cleavage site and N-linked glycosylation sites (Fig. 1). Based on the presence of the cleavage site, the processed LLPL would be predicted to consist of 379 amino acid residues, cor-
responding to a molecular mass of 43 kDa. This value is in agreement with the observed results following treatment of each recombinant LLPL with endoglycosidase F1. Based on amino-terminal sequence analysis of bovine brain LLPL, bovine LLPL is presumed to consist of 378 amino acid residues. These data support the interpretation that the precursor protein of LLPL is post-translationally modified by both signal peptide cleavage and N-linked glycosylation.

The observations that ACS has an acidic pH optimum and N-linked oligomannose co-localizes with β-hexosaminidase strongly indicate that ACS is a lysosomal enzyme. Deglycosylation of recombinant LLPLs had no effect on ACS activity. Thus, the oligomannose in ACS is probably involved in sorting ACS to lysosome.

The family of phospholipase A2 enzymes has expanded greatly in recent years (15). With this expansion, the criteria for recognition of an enzyme as a phospholipase A2 and its assignment to one of the 11 currently recognized groups have become more stringent. Currently, there are no lysosomal phospholipases A2 that meet such criteria. In 1997, a calcium-independent phospholipase A2 activity that was inhibited by serine hydrolase inhibitors was described (16). This enzyme was subsequently identified as a 1-cysteine peroxiredoxin (17); and thus, its characterization as a phospholipase A2 has been challenged.

We conclude that the product of the gene encoding LLPL is not a lysophospholipase, but ACS. Moreover, ACS is identified as a lysosomal enzyme and has the dual enzyme activities of a calcium-independent transacylase and phospholipase A2. A reaction mechanism for ACS is proposed (Fig. 10). In this model, the enzyme reacts with phospholipid and forms an acyl-enzyme intermediate at serine 165 of the mature protein based on the putative cleavage site. The acyl group of the intermediate is then transferred to a hydroxyl group of water or lipophilic alcohol such as ceramide.

We believe that ACS is a lysosomal phospholipase A2, but its
biological function is unknown. Several possible functions may be entertained, but remain to be tested. ACS may play a primary role in regulating the levels of ceramide within cells. Many investigators have postulated that ceramide may mediate cell differentiation and death responses. A means for regulating ceramide content, particularly through lysosome-mediated pathways, may be important in such a system. In this regard, ACS may function to terminate the biological activities of ceramide generated through signaling pathways perhaps by sequestering ceramide within lysosomes or by facilitating its movement across membrane leaflets. Alternatively, ACS produces a novel metabolite, 1-O-acylceramide. We have previously observed that phospholipids containing arachidonate can serve as the acyl donor for the transacylase reaction (2). It will be interesting to determine whether 1-O-arachidonoylceramide is a source for biologically active arachidonic acid in cells. Finally, it is also conceivable that water is the usual acceptor for ACS and that its primary function is to serve as a lysosomal phospholipase A2 with PE and PC as preferred substrates and their respective lysolipids and free fatty acids as products.

Based on the characterization reported in this study, the nomenclature used to refer to this lysosomal phospholipase is inaccurate. LCAT-like lysophospholipase does not describe the primary activity of this protein and should probably be discarded. Similarly, 1-O-acylceramide synthase may not reflect the primary lipid pathway catalyzed by the phospholipase because its in vivo activity has yet to be established. We propose therefore that the name lysosomal phospholipase A2 be used in reference to this enzyme.

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