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A turn in the road: How studies on the pharmacology of glucosylceramide synthase inhibitors led to the identification of a lysosomal phospholipase A_2 with ceramide transacylase activity

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A series of inhibitors of glucosylceramide synthesis, the PDMP based family of compounds, has been developed as a tool for the study of sphingolipid biochemistry and biology. During the course of developing more active glucosylceramide synthase inhibitors, we identified a second site of inhibitory activity for PDMP and its structural homologues that accounted for the ability of the inhibitors to raise cell and tissue ceramide levels. This inhibitory activity was directed against a previously unknown pathway for ceramide metabolism, *viz.* the formation of 1-*O*-acylceramide. In this pathway the addition of a fatty acyl group to the primary hydroxyl of ceramide occurs through a transacylation with either phosphatidylethanolamine or phosphatidylcholine as a substrate. However, both in the absence and presence of ceramide, water serves as an acceptor for the fatty acid. Thus the enzyme may be considered to be a phospholipase A_2. The enzyme is unique in that it has an acidic pH optimum and is localized to lysosomes by cell fractionation. More recently, the 1-*O*-acylceramide synthase has been purified, sequenced, and cloned. This phospholipase A_2 was discovered to be structurally homologous to lecithin cholesterol acyltransferase (LCAT). However, this phospholipase A_2 does not recognize cholesterol and lacks the defined lipoprotein-binding domain present in LCAT. We now refer to this enzyme as lysosomal phospholipase A_2 ($LPLA_2$). Although acidic phospholipase A_2 activities have been previously identified, $LPLA_2$ appears to be the first lysosomal PLA_2 to have been sequenced. This new phospholipase A_2 lacks an obvious and proven biological function.
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Introduction

Great strides have been made in our understanding of the biochemistry and biology of glycosphingolipids over the past 40 years. This is in no small part due to the contributions of Professors Robert Ledeen and Yoshitaka Nagai. It is therefore with great admiration that we are providing this review in their honor. Typical of their work and of many other investigators during this time is the observation that the study of glycosphingolipids has resulted in many unexpected insights into a diverse range of biochemical and cellular processes for sphingolipids. These phenomena include cell signaling [1], growth and differentiation [2,3], stress responses [4], and oncogenesis [5] to name a few. Our own work has primarily focused on the pharmacology of small molecule inhibitors of glucosylceramide synthase. These compounds, typified by 1-phenyl-2-decanolamino-3-morpholinopropanol (PDMP), have been used to probe the functions of glycosphingolipids [6]. In the course of developing these compounds and studying their specificity, we discovered that the PDMP homologues inhibited the metabolism of ceramide through a novel pathway. This pathway is the formation of 1-*O*-acylceramide.[1] This review traces the discovery of this pathway, its characterization, and the identification of the enzyme that catalyzes the formation of this unique sphingolipid.

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Ceramide metabolism and signaling pathways

Considerable experimental work was published establishing a key role for glycerolipids in cell signaling events long before

comparable functions were considered for sphingolipids. However, over the past fifteen years, a large corpus of work has demonstrated several potentially important roles for sphingolipids. These sphingolipids include the long chain bases such as sphingosine [7], sphingosine-1-phosphate [8], ceramide [9], ceramide-1-phosphate [10], and multiple glycosphingolipids. Among these sphingolipids, two have received particular attention as cell signaling molecules. These include sphingosine-1-phosphate and ceramide. The former lipid has been demonstrated to be the ligand for several members of the Edg receptor family [11]. Sphingosine-1-phosphate is also generated within cells. In both cases the primary effect is to promote cellular proliferation. The latter compound, ceramide, has been suggested to exert the opposite effect on cells, *viz.* growth arrest and apoptosis. A considerable number of publications support this hypothesis. Clearly, an understanding of the pathways for ceramide metabolism and the regulation of these pathways is important in proving the role that ceramide plays in these critical cellular events.

Ceramide is formed as part of the pathway for long chain base synthesis and acylation. It is also the key intermediate in the formation of most of the biologically important sphingolipids. Products of ceramide metabolism include sphingomeylin, cerebrosides (glucosylceramide and galactosylceramide), ceramide-1-phosphate, and ceramide-phosphoethanolamine. Less prominent pathways for ceramide metabolism also exist. Indeed, the formation of 1-*O*-acylceramide and more specifically the enzyme that catalyzes its synthesis is the subject of this review.

The formation of ceramide during cell signaling events is proposed to occur in one of two ways. First, ceramide may be generated through the activation of an acidic or neutral sphingomyelinase. Numerous agonists have been demonstrated to cause cellular ceramide accumulation through activation of sphingomyelin hydrolysis. Second, ceramide may be formed by *de novo* synthesis. A smaller number of agonists have been shown to stimulate cellular ceramide accumulation through the *de novo* pathway. Most examples of *de novo* ceramide synthesis are associated with cellular responses to stress or toxins leading to programed cell death. Recently it has been suggested that a therapeutic strategy for promoting cell death via ceramide would be to simultaneously block multiple pathways for ceramide metabolism [12]. Fully characterizing the alternative pathways for ceramide metabolism, understanding their relative contributions and subcellular localization would be prerequisites for exploiting this approach.

The phospholipase A_2 family of enzymes

The phospholipase A_2 superfamily is comprised of a broad range of enzymes that share the ability to hydrolyze the *sn*-2 ester bond of phospholipids [13]. The products of this reaction, free fatty acid and lysophospholipid, have important biological roles. The former is not only an important source of cellular energy, but is also the substrate for additional cellular messengers in the form of arachidonate metabolites. The latter products play signaling roles in addition to having important effects on membrane remodeling and membrane perturbation. Historically the phospholipase A_2s were thought to be small, secreted enzymes characterized by having a catalytic histidine, calcium dependence and disulfide rich polypeptides. The family of enzymes greatly expanded with the discovery of cytosolic PLA_2 activity without disulfide bonds and characterized by presence of a catalytic serine.

Currently there are eleven groups of phospholipase A_2s. For a candidate phospholipase A_2 to be categorized as a member of a novel group, four criteria should be met [14]. These include the ability to catalyze the hydrolysis of the *sn*-2 ester bond of a phospholipid, the identification of its complete amino acid sequence, the identification of all enzymes with identifiable sequence homology (paralogs being assigned subgroup letters), and the identification of splice variants.

Groups I, II, III, V, IX, X, and XI utilize a catalytic histidine and are of small molecular weight (13–15 kDa) [15]. Groups IV, VI, VII, and VIII utilize a neuclophilic serine and have little or no Ca^{2+} requirement for catalysis. The molecular weights are larger (26–114 kDa). Many members of these latter groups have C2 domains, are members of the α/β fold hydrolase proteins, and may contain a required Ser/His/Asp triad for activity [16].

Lysosomal phospholipase A_2 activities have been previously documented both within mammalian tissues and pathogens. The demonstration of the existence of a unique lysosomal phospholipase A_2 by the criteria outlined above has been more problematic. In 1997 the sequence of a protein was reported with Ca^{2+} independent phospholipase A_2 activity at pH 4.0 [17]. The activity of the putative lysosomal phospholipase A_2 was reported blocked by two serine hydrolase inhibitors. However, mutagenesis of the serine and cysteine had no effect on its phospholipase A_2 activity [18]. It was later demonstrated that this enzyme was a non-selenium glutathione peroxidase and not a phospholipase A_2 [19]. Thus until recently the structure and nature of the enzyme associated with the lysosomal phospholipase A_2 activity was not defined.

Discovery and characterization of the 1-*O*-acylceramide synthase pathway

Most mammalian glycosphingolipids are formed from the sequential addition of sugars to glucosylceramide. This cerebroside is formed from ceramide and UDP-glucose and is catalyzed by glucosylceramide synthase. Because glucosylceramide accumulates in Gaucher disease, it was hypothesized that inhibition of glucosylceramide synthase could be used as a strategy for the treatment of this and other glycosphingolipidoses [20]. An effort was undertaken to develop small molecule inhibitors of glucosylceramide synthase. D-*threo*-1-Phenyl-2-decanoylamino-3-morpholino-propanol (PDMP) was identified as a specific inhibitor of the synthase with an IC_{50} of 20 μM [21]. The other enantiomers, D-*erythro*, L-*threo*- and L-*erythro*, had no inhibitory activity against the synthase (Figure 1).

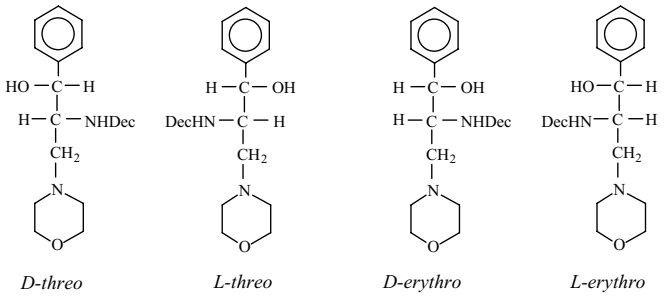

Figure 1. Structural enantiomers of PDMP.

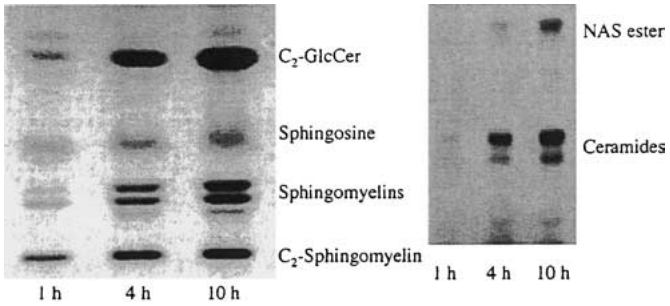

Figure 2. Products of *N*-acetylsphingosine metabolism in MDCK cells. MDCK cells (1.35×10^6) were seeded into 15-cm dishes in defined medium for 24 h. The medium was replaced with fresh medium containing 10 μM *N*-acetyl-[^{3}H]sphingosine (6.9×10^5 cpm/dish), and the cells were incubated for 1, 4, or 10 h. The total lipids were extracted and analyzed as previously reported. Reprinted with permission of The American Society of Biochemistry and Molecular Biology [23].

D-*threo*-PDMP was found to have dose limiting toxicity, however, and this was initially believed to be secondary to the accumulation of ceramide resulting from substrate accumulation. This interpretation was subsequently found to be incorrect when more active homologues of PDMP were developed.

Substitutions of a palmitoyl group for the decanoyl group and of a pyrrolidino group for the morpholino group resulted in a homologue of increased activity (IC_{50} ca. 1 μM) [22]. Careful study of this new homologue, D-*threo*-1-phenyl-2-palmitoylamino-3-pyrrolidino-propanol (P4), revealed that while glucosylceramide depletion occurred at a low concentration of inhibitor, ceramide accumulation was only present at 20-fold higher concentrations. Surprisingly, when a homologue was synthesized that replaced an aliphatic group for the phenyl group, inhibition of glucosylceramide synthesis was observed without ceramide accumulation. Furthermore, when the inactive enantiomers of P4 were tested, these compounds were inactive as glucosylceramide synthase inhibitors but comparably active in causing the accumulation of ceramide (Table 1). These results suggested that PDMP and P4 caused the accumulation of ceramide by inhibition of a second ceramide utilizing enzyme. A search was undertaken to identify this enzyme. Several known ceramide-utilizing enzymes were assayed including sphingomyelin synthase, acid and alkaline ceramidase, acid and neutral sphingomyelinase, and ceramide synthase. No inhibitory activity by P4 against any of these enzymes could be detected.

A different strategy was then employed. A short chain ceramide was synthesized that was radiolabeled in the long chain base [23]. This C2 ceramide was avidly metabolized to free sphingosine, long chain ceramides, and short and long chain sphingomyelin and glucosylceramide. However, another highly lipophilic compound was formed (Figure 2). This novel ceramide metabolite was structurally characterized as follows. The product was subjected to alkaline methanolysis yielding the substrate (*N*-acetylsphingosine). This finding suggested that the product represented an acylated ceramide with base sensitive acyl linkages. Two potential acylation sites were available, the 1 and 3 hydroxyl positions. Hydroxyls vicinal to a double bond are oxidized by 2,3-dichloro-5,6-dicyanobenzoquinone. Thus the 1 but not 3 hydroxyl of 1-*O*-acyl-*N*-acetylsphingosine should be susceptible to acylation following treatment with this compound. This was indeed found to be the case. When 1-*O*-acyl-*N*-acetylsphingosine was incubated with the quinone, the corresponding ketone was formed consistent with acylation at the 1-hydroxyl position. The novel sphingolipid was thus identified as 1-*O*-acylceramide.

The source of the fatty acid remained to be determined. Free fatty acid and fatty acylCoA were considered as potential substrates. Free fatty acids of varying chain length and saturation as well as fatty-acyl CoAs were inactive or inhibitory. However, upon the addition of liposomes incorporating phosphatidylcholine or phosphatidylethanolamine, a marked enhancement of activity was observed (Table 2). These data were consistent with the formation of 1-*O*-acylceramide through the transacylation with the phospholipid as substrate (Figure 3). The specificity of the reaction for *sn*-1 *versus sn*-2 fatty acids was next evaluated. When authentic phosphatidylethanolamine

Table 1. Effects of selected PDMP homologues on glucosylceramide synthase activity, ceramide and cell proliferation in MDCK cells

Compound	*Glucosylceramide synthase activity (percent inhibition)*	*Ceramide (percent control)*	*[^{3}H]Thymidine incorporation (percent control)*	*Cell protein (percent control)*
D,L-threo-P4 (4 μM)	86.5 $\pm$ 0.4	368	45.7 $\pm$ 5.5	34.8 $\pm$ 6.1
D,L-erythro-P4 (4 μM)	15.4 $\pm$ 1.4	438	51.5 $\pm$ 0.9	31.2 $\pm$ 5.7
1-Pyrrolidino-1-deoxyceramide (2 μM)	79.1 $\pm$ 0.2	100	100	101 $\pm$ 2.4

Table 2. Comparison of different acyl donors for transacylation of *N*-acetylsphingosine

	Transacylation activity (pmol/min/mg protein)			
Phospholipid concentration (μM)	*DOPC*	DOPC:PE	*DOPC:sulfatide*	*DOPC:PE:sulfatide*
1.28	15, 11			
12.8	40, 62	41, 43	93, 110	265, 261
128	18, 16	97, 59	288, 298	474, 486
640	0, 0	272, 268	95, 109	150, 144

The reaction mixture consisted of 47 mM sodium citrate (pH 4.5), 10 μM *N*-acetyl-[^{3}H]sphingosine, 30–60 ug/ml of MDCK cell supernatant, and 128 mM liposomal phospholipid. The molar ratios of the mixed liposomes were 70:30 for DOPC:PE, 86:14 for DOPC:sulfatide, and 61:25:14 for DOPC:PE:sulfatide. The incubation was for 20 min at 37°C. DOPC and PE denote dioctanoylphosphatidylcholine and phosphatidylethanolamine respectively.

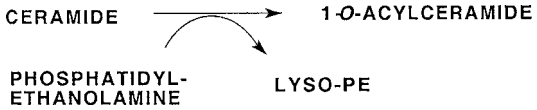

Figure 3. Proposed pathway for the synthesis of 1-*O*-acylceramide.

containing tritiated arachidonate in the *sn*-2 position was used as substrate, radiolabeled 1-*O*-acylceramide was formed. In addition, free arachidonate was released. This experiment provided the first suggestion that in the absence of ceramide as an acceptor, 1-*O*-acylceramide synthase could function as a phospholipase A_2.

In order to more definitively demonstrate that the activity in question was that of a phospholipase A_2, the 1-*O*-acylceramide synthase was purified [24]. A survey of rat tissues revealed that the activity was ubiquitously present; however, the specific activity was highest in the brain. The protein was, therefore, purified from bovine brain with a 193,000-fold increase in specific activity yielding a single band by Coomassie blue staining. Of note was a very significant enrichment of the protein by elution over a concanavalin A column with α-methyl mannoside elution, consistent with the enzyme being mannose rich.

The characterization of the purified enzyme revealed the following properties. The molecular mass was ca. 40 kDa and it was a glycoprotein of a single polypeptide chain. The purified enzyme had a pH optimum of 4.5. The divalent cations Ca^{2+} and Mg^{2+} enhanced but were not required for transacylase activity. The enzyme was neither activated nor inhibited in the presence of ATP or thiol reagents such as dithiothreitol and NEM. Thus the enzyme differs from groups I, II, and III phospholipase A_2s which are highly sensitive to such reagents. The phospholipase A_2 inhibitors bromoenollactone (BEL) and nonadecyltetraenyl trifluoromethyl ketone ($AACOF_3$) did not inhibit the enzyme activity. Thus inhibitors of both the calcium dependent and calcium independent cytosolic phospholipase A_2s of the higher molecular weights were inactive against the 1-*O*-acylceramide synthase. Importantly, in the absence of *N*-acetylsphingosine, the enzyme acted as a phospholipase A_2 using water and not *N*-acetylsphingosine as an acceptor (Figure 4).

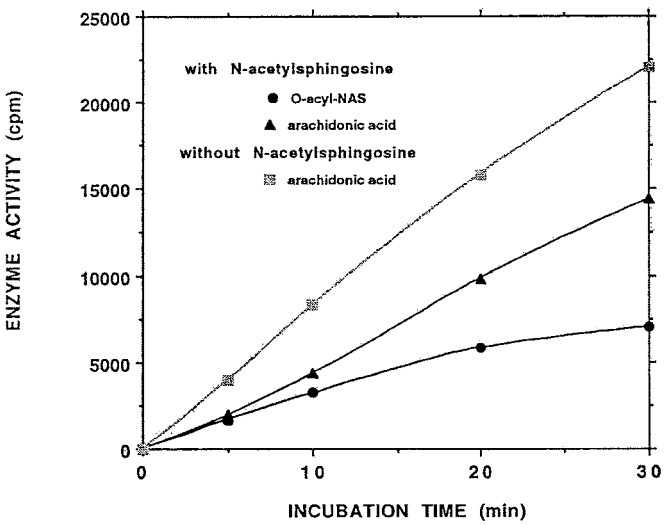

Figure 4. Dual enzyme activity of 1-*O*-acylceramide synthase. The transacylase and phospholipase A_2 activities of 1-*O*-acylceramide synthase purified from bovine brain were measured. The reaction was conducted using liposomes containing L-α-1-acyl-2-[1-^{14}C]arachidonoyl-phosphatidylethanolamine in the presence or absence of 10 μM *N*-acetylsphingosine as previously reported [24]. Free aracidonic acid was released in the absence of *N*-acetylsphingosine. Both free arachidonic acid and 1-*O*-acylceramide were formed in the presence of *N*-acetylsphingosine. Reprinted with permission of The American Society of Biochemistry and Molecular Biology [24].

Cloning and further characterization of 1-*O*-acylceramide synthase

The purified protein was subjected to tryptic digestion and the fragments were sequenced by mass spectrometry [25]. A BLAST search revealed that the partial amino acid sequences were highly homologous to the amino acid sequence deduced from the human cDNA encoding a protein termed LCAT-like lysophospholipase (LLPL) [26]. Several ESTs were identified following a T BLAST N data search of the GenBank/EBI Data Bank, including bovine ESTs. Primers were subsequently designed and the entire coding sequences for the bovine and mouse coding sequences were obtained. The entire coding sequences

of the mouse, bovine, and human genes were obtained from the respective kidney total RNA by RT-PCR using primer sequences designed on the basis of the EST sequences. The assigned GenBank accession numbers of the mouse and bovine sequences are AF468958 and AY072914 respectively.

A comparison of the deduced amino acid sequences from the human, mouse, and bovine genes was made. The human and mouse cDNAs encode 412 amino acid polypeptides with an acidic isoelectric point. The bovine cDNA encodes a 407 amino acid polypeptide with a basic isoelectric point. A predicted signal peptide sequence exists for all three polypeptides. In addition, there are several putative N-linked glycosylation sites (three in the bovine protein, four in the mouse and human proteins). There is also a putative lipase motif, AXSXG, predicted for each protein. This motif is also found in *Bacillus*.

In order to prove that the derived sequence encoded for 1-*O*-acylceramide synthase activity, the entire open reading frame of the mouse gene was cloned into the *HindIII* and *XhoI* sites of pcDNA3 to generate carboxyl-terminally tagged proteins with or without FLAG, HA, or c-Myc peptides. The vectors were transfected into COS-7 cells. Both 1-*O*-acylceramide synthase and phospholipase A_2 activities were observed in each transfected cell preparation. The presence of the carboxyl terminal tag had no effect on activity. Immunoprecipitates of the tagged proteins revealed that the phospholipase and transacylase activities were intrinsic to the expressed proteins and that these did not represent a cofactor or activator of another enzyme (Figure 5).

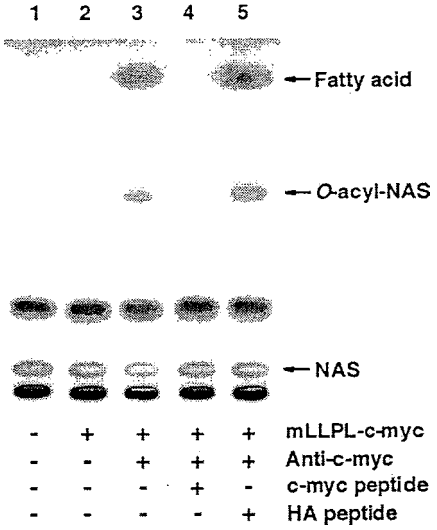

Figure 5. Demonstration that LLPL has both transacylase and phospholipase activities. Cells were transfected with carboxyl-terminally c-Myc-tagged mouse LLPL. LLPL was recovered with an anti-c-Myc monoclonal antibody in the presence or absence of c-Myc or hemaglutinin peptide. The transacylase activity was measured in soluble cell fraction as previously described. Reprinted with permission of The American Society of Biochemistry and Molecular Biology [25].

Taniyama et al. [26] reported that human recombinant LLPL has lysophospholipase activity. Cells transfected with the mouse synthase demonstrated no lysophospholipase activity under the conditions employed by these investigators at either acidic or neutral pH. Because the deduced amino acid sequence of the coding region of the gene is 49% homologous with lecithin cholesterol acyl transferase (LCAT), we studied whether the expressed synthase has any LCAT activity. Although marked phospholipase A_2 activity was observed in the presence of cholesterol and phosphatidylcholine, no LCAT activity was observed. Thus although both 1-*O*-acylceramide synthase (LLPL) and LCAT have phospholipase A_2 and transacylase activities, they recognize different acceptors. A review of the predicted secondary structures of the two proteins reveals that they are structurally homologues near their catalytic domains, but vary markedly at the cholesterol and lipoprotein binding domains of LCAT. A recently published dendrogram also reveals a distant relationship to phospholipid:diacylglycerol acyltransferase.

Based on the submission of the cDNA sequence to the NCBI by Taniyama *et al*. the human gene has been assigned the abbreviation LYPLA3 for lysophospholipase 3 [26]. Given that the expressed protein has no detectable lysophospholipase activity, this terminology should probably be changed. Gene mapping reveals a location at 16q22.2. LCAT maps to 16q22.1, and there are currently six genes mapped between LCAT and LYPLA3. The mapping data suggests that LYPLA3 may have arisen as a gene duplication in the remote past.

The localization of 1-*O*-acylceramide synthase has also been evaluated. The enzyme activity was assayed in MDCK cell homogenates that were subjected to Percoll gradient fractionation. Under these conditions the enzyme activity co-localized with that of β-hexosaminidase. This is consistent with a lysosomal localization of the enzyme.

In order to demonstrate that the AXSXG motif was the site of the catalytically active serine, a serine-to-alanine substitution was made using the overlap extension method. The soluble fraction from cells transfected with the mutated construct failed to show any phospholipase A_2 or transacylase activities. Western blots revealed, however, that the mutated protein was expressed at levels to that of the 1-*O*-acylceramide synthase (Figure 6). This observation is consistent with the view that the serine residue in the AXSXG motif is an active site and that the enzyme forms an acyl-enzyme intermediate via the hydroxyl group of serine. In addition, the catalytic triad of serine 181, aspartic acid 345, and histidine 377 observed in LCAT is also found in the 1-*O*-acylceramide synthase. This triad is more generally characteristic of the α, β hydrolase fold proteins.

Thus 1-*O*-acylceramide synthase (LLPL) appears to fulfill several criteria for being defined as a unique phospholipase A_2. It is capable of hydrolyzing fatty acids from the *sn*-2 position of both phosphatidylcholine and phosphatidylethanolamine. The complete amino acid sequence has been defined. Like several other phospholipase A_2s, 1-*O*-acylceramide synthase contains

Table 3. Comparison of 1-*O*-acylceramide synthase ($LPLA_2$) to cloned high molecular weight PLA_2s

Group	*Common name*	*Species*	*Size (kDa)*	*Chromosome*	*sn-2 fatty acid preference*	*Calcium requirement*	*Activity profile*	*Expression profile*
IVA	$cPLA_2$-α	All	85	hu 1	AA	Yes	$AACOF_3$ sensitive	Ubiquitous
IVB	$cPLA_2$-β	Human	100	hu 15	None	Yes	ND	Ubiquitous
IVC	$cPLA_2$-γ	Human	55	hu 19	AA	No	ND	Ubiquitous
VIA	$iPLA_2$-β	All	60	hu 22	None	No	BEL, ATP sensitive	Ubiquitous
VIB	$IPLA_2$-γ	Human	80–88	hu 7	None	No	BEL, ATP sensitive	Heart, brain, placenta
	$LPLA_2$, ACS, LLPL LYPLA3	Mouse, cow, human	40	hu 16	Yes	No	$AACOF_3$, BEL, and ATP insensitive	Ubiquitous

This table excludes group VII and VIII phospholipase A_2s that are also PAF acetyl hydrolases.

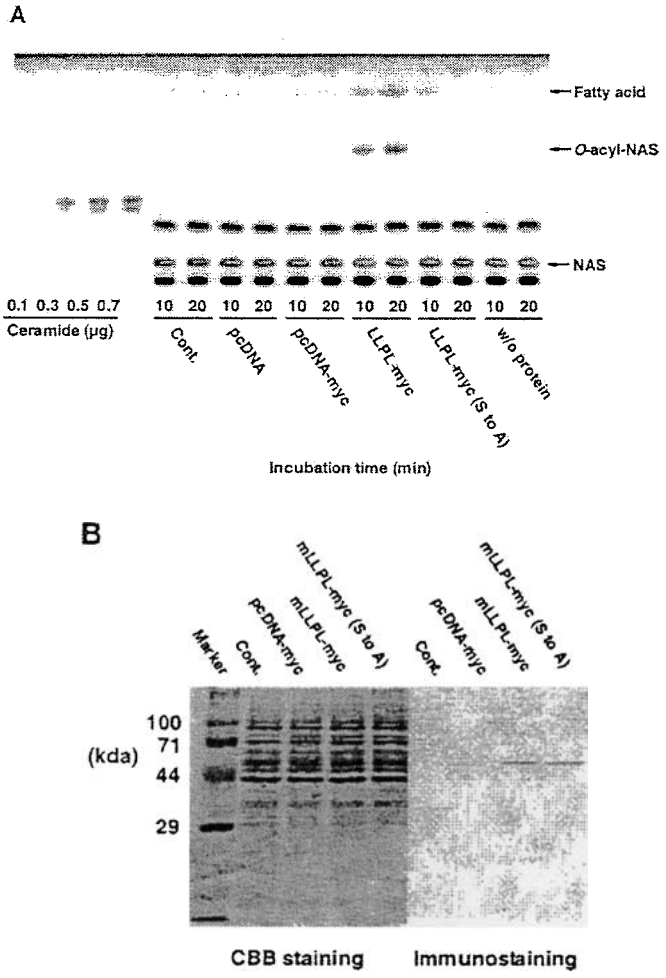

Figure 6. Expression of mouse LLPL and mutated LLPL in COS-7 cells. COS-7 cells were transiently transfected with pcDNA3, pcDNA3-c-Myc-tagged mouse LLPL, or pcDNA3-cMyc-tagged mutated mouse LLPL. The soluble fractions, obtained from the transfected cells, were assayed for transacylase activity (A) or Western blotting (B) as previously described. Reprinted with permission of The American Society of Biochemistry and Molecular Biology [25].

a catalytically active serine. Mutagenesis of this site abolishes the phospholipase A_2 activity. It is phylogenetically related to a larger family of lipases, including LCAT. However, unlike other groups of phospholipase A_2s, the new phospholipase A_2 has an acidic pH optimum, is mannose rich, and is localized to lysosomes. The phospholipase has a unique activity profile and chromosomal location. A comparison to other high molecular weight phospholipase A_2s is provided in Table 3.

Conclusions and future questions

The work reviewed in this paper can be summarized as follows. First, enantiomers of the glucosylceramide synthase inhibitors PDMP and P4 are equally active at raising cell ceramide levels by inhibition of a pathway that does not involve glucosylceramide synthesis. Second, this pathway is novel and consists of the formation of 1-*O*-acylceramide through the transacylation of ceramide at the 1-hydoxyl position. The fatty acid is donated from the *sn*-2 position of phosphatidylethanolamine or phosphatidylcholine. Third, by purification the transacylase is mannose rich and active at an acidic pH optimum. In the absence of ceramide, water may be used as an acceptor. Therefore the enzyme acts as a phospholipase A_2. Fourth, sequence analysis reveals that the enzyme is 49 percent identical to LCAT and is a member of the α,β-hyrolase fold family of enzymes. The human gene is identical to a previously described gene names LLPL for LCAT-like lysophospholipase. However, the expressed protein lacks significant lysophospolipase activity. Fifth, the enzyme can be localized to lysosomes by cell fractionation. Sixth, it contains a critical serine at the catalytic site. It is calcium independent and is not inhibited by $AACOF_3$ or BEL. 1-*O*-Acylceramide synthase is, therefore, a novel lysosomal phospholipase A_2.

Several questions emerge from these observations and remain the subject of ongoing studies. We believe that two questions are particularly significant. First, is the formation of 1-*O*-acylceramide a biologically important pathway or is water the preferred acceptor for the lysosomal phospholipase A_2?

While it has been possible to measure endogenous levels of 1-*O*-acylceramide, the levels of this sphingolipid are very low compared to those of ceramide or free sphingosine. In addition, other alcohols serve as acceptors for the synthase and thus other acylated alcohols may occur naturally. Finally, the hydrolysis of phosphatidylcholine or phosphatidylethanolamine with the formation of free fatty acid may be the biologically relevant pathway. We anticipate that the development of *in vitro* and *in vivo* models with the elimination or overexpression of the lysosomal phospholipase A_2 activity will clarify this issue.

Second, what is the biological role of the lysosomal phospholipase A_2? Little work has been done on defining the functional significance of lysosomal phospholipase A_2 activity. Two approaches, however, are intriguing and potentially important. The first and more obvious approach has been the consideration of a role for lysosomal phospholipase A_2 on the metabolism of *endogenous* phospholipids. Fisher *et al.* have studied one example of this type of activity. They have argued that an alveolar macrophage lysosomal phospholipase A_2 is critical for surfactant turnover [27]. A model for the study of this activity is the rat lung in which dipalmitoylphosphatidylcholine is the major phospholipid component. The metabolism of this lipid is dependent on a lysosomal enzyme with phospholipase A_2 activity and an acidic pH optimum. A second and less obvious consideration would be a role for lysosomal phospholipase A_2 in the metabolism of *exogenously* incorporated phospholipids that might occur via the phagocytosis of microbes. When macrophages are exposed to mycobacteria, a lysosomal phospholipase A_2 activity is required for the metabolism of cardiolipid within the cell wall [28]. This has been proposed to be important for host defense and the prevention of proliferation of the mycobacteria within the phagosome. While these are intriguing clues to the potential biological role of the lysosomal phospholipase A_2, the ubiquitous expression of the enzyme suggests that there are functions for this phospholipase that extend beyond the macrophage.

Note

1. For historical accuracy, the enzyme described in this paper, 1-*O*-acylceramide synthase, has been given several names with associated abbreviations. For cogency, these are listed as follows: ACS, 1-*O*-acylceramide synthase; LLPL, LCAT-like lysophospholipase; LYPLA3, lysophospholipase 3; LPLA2, lysosomal phospolipase A_2.

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