mRNA Encoding a New Lipolytic Enzyme Expressed in Rabbit Lacrimal Glands

Susann G. Remington¹ and J. Daniel Nelson¹,²

PURPOSE. In previous work, lacrimal glands of female mice were shown to express an mRNA encoding pancreatic lipase-related protein 1 (PLRP1), a member of the triacylglycerol lipase family. To investigate the hypothesis that lacrimal glands express mRNAs encoding other lipolytic enzymes, the present study was conducted to look for triacylglycerol lipase-related mRNAs in the lacrimal glands of rabbits.

METHODS. Degenerate polymerase chain reaction (PCR) primers were designed based on two conserved amino acid motifs, RITGLD, and DI(F/P)Y/FPN, in the triacylglycerol lipase family. Lacrimal gland cDNAs were amplified, and the expected 200-bp products were subcloned and sequenced. One product encoded a new lipase-related sequence, which was termed lacrimal lipase (LL). The complete cDNA sequence of rabbit LL was determined, and labeled LL cDNA was used to probe RNA blots of several rabbit tissues and a genomic DNA blot.

RESULTS. BLAST database searches indicated that the predicted amino acid sequence of LL is related to phosphatidylethanolamine phospholipase A2 (PS-PLA2) and to members of the triacylglycerol lipase family. Labeled LL cDNA hybridized to a 2.1-kb message in RNA blots of rabbit lacrimal gland, harderian gland, heart, liver, and pancreas. These blots demonstrated no gender-based differential expression in rabbit lacrimal or harderian glands. Hybridization of labeled LL cDNA to a genomic DNA blot suggested that LL was a single-copy gene.

CONCLUSIONS. Male and female rabbit lacrimal glands expressed an mRNA encoding LL, a new predicted member of the triacylglycerol lipase family. (Invest Ophthalmol Vis Sci. 2002;43: 3617–3624)

The preocular tears lubricate and protect the ocular surface epithelia. The tears are composed of an aqueous phase containing many proteins secreted by the main and accessory lacrimal glands; mucins supplied by the corneal epithelial, conjunctival epithelial, and goblet cells; and a thin lipid layer secreted by the meibomian glands of the eyelids. (The harderian glands of nonprimate vertebrates also secrete tear lipids.) Tear lipids provide an evaporation barrier at the air–aqueous interface to modify tear lipids. Having found evidence for the expression of a lipolytic enzyme in the lacrimal glands (PLRP1 in female mouse), we decided to use degenerate polymerase chain reaction (PCR) primers to look for the expression of mRNAs encoding other lipolytic enzymes, specifically triacylglycerol lipases, in the lacrimal glands of rabbits. To identify highly conserved amino acid segments of the lipase family suitable for degenerate primer design, we examined an amino acid sequence alignment of the three major subfamilies of triacylglycerol lipases: lipoprotein lipase, hepatic lipase, and PLA2. PLA2 mRNA has been localized to the acinar cells of lacrimal glands.

In previous work, we investigated the differential expression of mRNAs between female and male mouse lacrimal glands. We found that the acinar cells of female mouse lacrimal glands expressed an mRNA encoding pancreatic lipase–related protein 1 (PLRP1). However, male mouse lacrimal glands did not express detectable levels of PLRP1 mRNA (nor did the lacrimal glands of rats or rabbits of either gender). PLRPs may also be expressed in human lacrimal glands. Recently, peptide fragments of PLRP1 and PLRP2 have been identified in a human tear sample by mass spectrometry (Fung K, University of Colorado Health Sciences Center, Denver, CO, personal communication, 2000). Based on sequence attributes, PLRP1 is a member of the triacylglycerol lipase family of enzymes.

Lipolytic enzymes in ocular tears could act at the lipiddaqueous interface to modify tear lipids. Having found evidence for the expression of a lipolytic enzyme in the lacrimal glands (PLRP1 in female mouse), we decided to use degenerate polymerase chain reaction (PCR) primers to look for the expression of mRNAs encoding other lipolytic enzymes, specifically triacylglycerol lipases, in the lacrimal glands of rabbits. To identify highly conserved amino acid segments of the lipase family suitable for degenerate primer design, we examined an amino acid sequence alignment of the three major subfamilies of triacylglycerol lipases: lipoprotein lipase, hepatic lipase, and pancreatic lipases. We modeled degenerate PCR primers after two conserved amino acid motifs and used the primers to screen lacrimal glands of rabbits for the expression of related lipase mRNAs. We found that rabbit lacrimal glands produced an mRNA encoding a new sequence, which was related to the triacylglycerol lipase family. We called this sequence lacrimal lipase (LL), because lacrimal gland was our original tissue source for the mRNA.

MATERIALS AND METHODS

Animals

Animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Lacrimal gland, harderian gland, liver, and pancreas were dissected from New Zealand rabbits, and heart tissue was dissected from Dutch Belted rabbits. The same tissues were also dissected from Sprague-Dawley rats. Tissues were frozen in liquid nitrogen and stored at −70°C until needed.
Nucleic Acid Isolations

Poly(A)^+ RNA was isolated from approximately 125 mg of each tissue in a single-step procedure (PolyATtract System 1000; Promega, Madison, WI) with modifications for the hardier glands.

Genomic DNA was isolated from New Zealand rabbit liver^10 and dialyzed against several changes of TE (10 mM Tris-HCl [pH 7.6] and 1 mM EDTA).

Degenerate Primer PCR

For first-strand cDNA synthesis, 3 to 4 μg poly(A)^+ RNA was denatured with 0.4 μg oligo(dT)_{12-18} at 65°C for 3 minutes. cDNA synthesis reactions were performed in 50-μL volumes at a final concentration of 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol, and 1 mM each deoxyribonucleic acid triphosphate (dNTP), in the presence of 50 U RNasin Rnase inhibitor and 400 U Moloney murine leukemia virus (MLMV) reverse transcriptase (Promega) at 37°C for 1 hour. The cDNA was precipitated with ammonium acetate and dissolved in 50 μL water.

Degenerate polymerase chain reaction (PCR) primers were designed based on two highly conserved amino acid sequence motifs in the triacylglycerol lipase family of enzymes. The amino acid motifs were chosen by visual inspection of an amino acid alignment of lipoprotein lipase, hepatic lipase, and pancreatic lipases from several different species. The upstream primer (5'-CGGATCCGNNTHACNG-GNYTNGAYSC-3') corresponded to positions 183 to 189 of the amino acid alignment (RITGLD(P/A)); the downstream primer (5'-CGGATCCNCCRRTTNGGRWADAWRTC-3') corresponded to positions 237 to 243 of the amino acid alignment (D(I/F)(Y/F)PNGG) (Genosys Biotechnologies, Woodlands, TX). Each degenerate primer contained a 5^' restriction enzyme site.

PCR amplification was performed with 2.5 μL of each cDNA synthesis reaction in a 25-μL volume at a final concentration of 10 μM Tris-HCl (pH 8.3), 50 mM KCl, 1.8 mM MgCl₂, 200 μM each dNTP, and 4 μM of each primer, with 2 U of DNA polymerase (AmpliTaq; Applied Biosystems, Inc., Foster City, CA). The reaction mixtures were denatured at 95°C for 1 minute, and then cycled 40 times in a DNA thermal cycler (GeneAmp System 2400; Applied Biosystems, Inc.) as follows: 95°C for 30 seconds, 55°C for 1 minute, 72°C for 2 minutes. Ramping in the first five cycles was slowed to 40% between the annealing temperature of 55°C and the extension temperature of 72°C; the last cycle was extended at 72°C for an additional 5 minutes.

PCR products of approximately 200 bp in length were extracted from 1.7% agarose gels with a kit (GeneClean II Kit; Bio 101, Vista, CA) and cloned into the plasmid vector pCR2.1 (Invitrogen, Carlsbad, CA).

Rapid Amplification of cDNA Ends-PCR

The complete cDNA sequence of LL was obtained using the rapid amplification of cDNA ends (RACE) technique. In 5' RACE-PCR, poly(A)^+ RNA from rabbit lacrimal gland was initially primed with the antisense oligonucleotide 5'-TCCCTTATAGCCAGTGCGTC-3' (Genosys Biotechnologies), complementary to bases 725 to 744 of the LL cDNA sequence (Fig. 1), and cDNA was synthesized with avian myeloblastosis virus (AMV) or MLMV reverse transcriptase (Promega). Single primer PCR yielded a 5' 375-base extension of LL, with the sequence specific oligonucleotide primer 5'-GGGATTCGATGATCCAC-3', complementary to bases 692 to 711 of the LL cDNA sequence (Fig. 1) and flanked by an EcoRI restriction enzyme site. This PCR product was identified in an ethidium bromide stained agarose gel.

An additional primer was needed to obtain the 5' end of rabbit LL. Using the newly generated 5' LL extension, we designed a sequence-specific oligonucleotide primer 5'-CTGGAATTCCTGGAAGCGGACG-3', complementary to bases 319 to 336 of the rabbit LL cDNA sequence (Fig. 1). The primer adapters were as follows: 5'-GACTCGAGGATCAAGC(T)_{1-3}' and 5'-GACTCGAGGATCAAGC(T)_{1-3}'.

In 3' RACE-PCR, poly(A)^+ RNA from rabbit lacrimal gland was primed with adapter primer 5'-GACTCGAGGATCAAGC(T)_{1-3}' for cDNA synthesis with MLMV reverse transcriptase (Promega). The sequence-specific oligonucleotide primer was within a 5' extension to the original LL fragment: 5'-TGGGATTAAGTCTAGGAGCCG-3', corresponding to bases 553 to 572 of the LL cDNA sequence (Fig. 1).

DNA Blots of PCR Products

PCR products were electrophoresed in 1% agarose gels in 1× TBE (90 mM Tris base, 90 mM boric acid, 2 mM EDTA); ethidium bromide-stained DNA standards were photographed, and the nucleic acid products were capillary transferred to nitrocellulose (Schleicher & Schuell, Keene, NH). Prehybridizations and overnight hybridizations were in 50% formamide at 42°C. The probe for 5' RACE-PCR was the 5' single primer PCR extension product, representing bases 262 to 711 of the rabbit LL cDNA (Fig. 1). The probe for 5' RACE-PCR was the original degenerate primer PCR fragment, representing bases 657 to 760 of the rabbit LL cDNA (Fig. 1). The DNA probes were random prime labeled with [α-32P]dCTP (NEN Life Science Products, Boston MA) using a labeling system (Prime-a-Gene Labeling System; Promega). The labeled probes were denatured in a boiling water bath for 5 minutes and added to fresh hybridization solution at 1 to 4 × 10^6 counts per minute (cpm)/ml for overnight incubations.

The blots were washed several times in 1× SSC, 0.1% SDS at room temperature and finally in 0.2× SSC and 0.1% SDS at 55°C and exposed to x-ray film (XAR; Eastman Kodak Co., Rochester, NY).

Cloning and Sequencing

PCR products were extracted from agarose gels and cloned into the plasmid vector pCR2.1 (Invitrogen). The 1.5-kb 3' RACE-PCR product was also cloned into a vector (pBluescript II SK+; Stratagene, La Jolla, CA) for bidirectional exonuclease III deletion analysis using the commercial system (Erase-a-Base; Promega). Double-stranded plasmid inserts were sequenced by one of two systems (Silver Sequence DNA Sequencing System; Promega; or the T7 Sequenase 2.0 sequencing kit; Amersham Pharmacia Biotech, Piscataway, NJ). The cDNA sequence of rabbit LL (Fig. 1) was derived from completed composite sequences in each direction.

Sequence Analyses

Sequence information was organized by computer (GeneWorks suite of computer programs; IntelliGenetics, Campell, CA). A signal peptide cleavage site was predicted by using SignalP, a Web-based program at the Center for Biological Sequence Analysis (provided in the public domain by the Technical University of Denmark, Lyngby, Denmark, and available at http://www.cbs.dtu.dk/services/SignalP/). Sequences were compared with those in the GenBank databases, using the BLAST programs (GenBank and BLAST are provided in the public domain by the National Center for Biotechnology Information [NCBI], Bethesda, MD, and are available at http://www.ncbi.nlm.nih.gov/Genbank and http://www.ncbi.nlm.nih.gov/BLAST/). A phylogenetic tree was constructed using Clustal W to align the sequences, and the cluster algorithm of the Phylogenetic Tree Web-based program at GeneBee (provided in the public domain by the A. N. Belozersky Institute of Chemical Biology, Moscow State University, Moscow, Russia, and available at http://www.genebee.msu.su).

RNA Blots

Poly(A)^+ RNA samples, 2 μg per lane, were denatured in 50% formamide and 6% formaldehyde in low-ionic-strength buffer (20 mM phos-
phate, pH 7.7) at 65 °C for 5 minutes. The samples were electrophoresed through a 1% agarose gel containing 6% formaldehyde in 20 mM phosphate buffer (pH 7.7). RNA standards (Life Technologies, Rockville, MD) were visualized by staining a portion of the gel with 0.5/10^6 g/mL ethidium bromide. The RNA in the remainder of the gels was capillary transferred to nylon membranes (GeneScreen; NEN Life Science Products) using 10/10^6 SSC (1.5 M NaCl, 0.15 M sodium citrate, pH 7.0). Prehybridizations and overnight hybridizations were in 50% formamide at 42 °C according to the manufacturer’s protocol.

**FIGURE 1.** Nucleic acid sequence and deduced amino acid sequence of rabbit LL. The underscored amino acids correspond to the degenerate primers used for PCR. Arrow: the predicted signaling peptide cleavage site. These sequence data are available under GenBank accession number AF551188.

The LL probes were the 1.5-kb, 3'RACE-PCR fragment, representing bases 553 to 1989 of the rabbit LL cDNA and the 350-bp, 5'RACE-PCR fragment representing bases 1 to 336 (Fig. 1). The actin probe was a 1.5-kb mouse β-actin fragment (Stratagene). These DNA probes were random prime labeled with [32P]-32P]dCTP (NEN Life Science Products) using a labeling system (Prime-a-Gene; Promega). The labeled probes were denatured in a boiling water bath for 5 minutes and added to fresh hybridization solution at 1 to 6/10^6 cpm/mL for overnight incubations.

Lipolytic Enzyme mRNA in Lacrimal Glands 3619

**poly(A)**

The LL probes were the 1.5-kb, 3'RACE-PCR fragment, representing bases 553 to 1989 of the rabbit LL cDNA and the 350-bp, 5'RACE-PCR fragment representing bases 1 to 336 (Fig. 1). The actin probe was a 1.5-kb mouse β-actin fragment (Stratagene). These DNA probes were random prime labeled with [32P]-dCTP (NEN Life Science Products) using a labeling system (Prime-a-Gene; Promega). The labeled probes were denatured in a boiling water bath for 5 minutes and added to fresh hybridization solution at 1 to 6×10^6 cpm/mL for overnight incubations.
The blots were washed several times in 2× SSC, 1% SDS at 60°C (55°C for mouse actin) and finally in 0.2× SSC at room temperature. The blots were exposed to x-ray film (XAR film; Eastman Kodak Co.) with an intensifying screen at −70°C. After the LL hybridizations, the blots were stripped in boiling 0.1× SSC and 1% SDS for 20 minutes and hybridized with labeled o-actin.

Genomic DNA Blot

Genomic DNA, 10 μg per sample, was digested overnight with the following restriction endonucleases: Apal, BamHI, BglII, EcoRI, HindIII, Kpnl, NcoI, NdeI, PstI, SacI, and Spel (New England Biolabs, Beverly, MA). The samples were electrophoresed through a 0.7% agarose gel in 1× TBE at 1 V/cm for 24 hours, ethidium bromide-stained standards were photographed, and the DNA was capillary transferred to nitrocellulose (Schleicher & Schuell). Prehybridizations and overnight hybridizations were in 50% formamide at 42°C.

The probe, representing bases 657 to 760 of rabbit LL (Fig. 1), was random prime-labeled with (α-32P)dCTP (NEN Life Science Products) using the labeling system (Prime-a-Gene Labeling System; Promega), denatured, and incubated overnight in fresh hybridization solution at 1 × 10⁶ cpm/mL. The blots were washed several times in 1× SSC, 0.5% SDS at room temperature and finally in 0.2× SSC, 1% SDS at 60°C, and exposed to x-ray (XAR; Eastman Kodak Company).

RESULTS

New Lipase Sequence in Lacrimal Glands

To look for the expression of triacylglycerol lipase-related mRNAs in the lacrimal and hardierian glands of rabbits, we used degenerate primer PCR. We designed primers based on two conserved amino acid motifs in a sequence alignment of triacylglycerol lipases. We chose amino acid positions 185 to 189 (RITGLD(P/A)) for the 5′ primer and 237 to 243 (DI(IF)(Y/F)PNGG) for the 3′ primer, because the corresponding nucleic acid sequences could be rendered with minimal degeneracy. Each amino acid motif was common to the three vertebrate subfamilies of triacylglycerol lipases: lipoprotein lipase, hepatic lipase, and pancreatic lipases. The primer sequences were nearly preserved in the related Drosophila yolk proteins as well. These primers should identify known members of the triacylglycerol lipase family. They also had the potential to identify previously unknown mRNAs that share these two conserved sequence motifs.

In control experiments, we verified that the degenerate primers amplified the expected triacylglycerol lipase sequence fragments from control tissues of rabbits and rats: hepatic lipase from liver, lipoprotein lipase from heart, and pancreatic lipases from pancreas (data not shown).

Next, we amplified cDNAs prepared from rabbit, rat, and mouse lacrimal and hardierian glands. Degenerate primer PCR yielded prominent 200-bp products from lacrimal and hardierian gland cDNAs; omitting the cDNA in control reactions resulted in no amplified product. We subcloned and sequenced the 200-bp products, and compared the sequences with those in the GenBank databases, using BLAST. Our screen identified several known triacylglycerol lipases, lipoprotein lipase, endothelial cell-derived lipase, pancreatic lipase and PLRP1. Our screen also identified a new lipase-related sequence fragment in rabbit and rat lacrimal glands which, until January 2002 (see later description), did not match any entries in the GenBank nonredundant (nr) database. BLAST comparisons of the predicted amino acid sequence of the fragment with those in the protein databases indicated that the fragment was related to triacylglycerol lipases. We refer to the new sequence as LL, denoting lacrimal lipase.

Full-Length LL Sequence

To obtain the complete cDNA sequence for rabbit LL, we used RACE-PCR. We were unsuccessful in applying the standard 5′ and 3′ RACE protocols to the 124 known bases of the original LL internal fragment (bases 657 to 760; Fig. 1). In these initial RACE-PCR trials, many of the nonspecific products resulted from double priming by the adapter primers. We reasoned that if we supplied only a single sequence-specific primer in a PCR reaction, products may include partial extensions of the LL sequence, primed at the intended position and at a nonspecific location. Using one of the 5′ antisense primers (see the Materials and Methods section), this approach succeeded in identifying a partial 5′ extension of LL (bases 262 to 711; Fig. 1). The single-primer PCR approach did not succeed in identifying a 3′ extension.

Next, we used the extended LL sequence (bases 262 to 636; Fig. 1) to design new sequence-specific 5′ and 3′ RACE-PCR primers (GeneWorks, IntelliGeneics). To identify PCR products that represented longer extensions of LL, we blotted the gel-separated products to nitrocellulose and probed them with labeled fragments of LL. Using this approach, we identified one full-length 5′ extension product and one complete 3′ product. (See Materials and Methods for the sequences of the successful RACE-PCR oligonucleotide primers.) We subcloned and sequenced these RACE-PCR products in both directions, and constructed a composite LL cDNA sequence (Fig. 1).

The 2-kb nucleic acid sequence of LL contains a 452-amino-acid open reading frame, which predicts a 51-kDa protein. By analogy with related lipase sequences, LL may be a secreted protein. SignalP, the Web-based program described earlier, predicts a signal peptide cleavage site after amino acid 16 (Fig. 1), implying that the rabbit LL mRNA may encode a 49-kDa secreted enzyme.

Human Sequences Similar to Rabbit LL

We used BLAST to compare the rabbit LL sequence with those in the NCBI databases. Comparison of the predicted amino acid sequence of LL with translations of the nucleic acid sequence entries revealed significant similarity to phosphatidylserine phospholipase A1 (PS-PLA1; expectation value, 4e-45) and to a variety of triacylglycerol lipase family members, represented by PLRP2 in an alignment (Fig. 2A). The predicted amino acid sequence of rabbit LL (residues 55 to 283) was 42% identical with human PS-PLA1, and 40% identical with human PLRP2. While this manuscript was under review, two entries representing the human orthologue of LL were posted in GenBank: accession numbers AY036912 and AK074229. Sequence AY036912 was recently reported to be a membrane-associated phosphatidic acid-selective phospholipase A1 (mPAPLA1).

We generated a phylogenetic tree showing the potential molecular evolutionary relationship of rabbit LL with other members of the triacylglycerol lipase family (Fig. 2B). LL is most similar to PS-PLA1 and more distantly related to the other triacylglycerol lipases found on neighboring branches of the tree.

In the human genomic sequence database, BLAST aligned the nucleic acid sequence of rabbit LL with a single, putative human orthologue on chromosome 3 (accession no. Hs3p27:7075; data not shown). Rabbit LL is approximately 90% identical with the corresponding exons of the human chromosomal sequence.

BLAST also identified several related (85–90% identity) expressed sequence tags (ESTs) in the human EST database. These data imply that the human orthologue of LL may be expressed in normal and tumor tissues of human stomach, colon, pancreas, skin, prostate, and ovary (accession nos.
The rabbit LL sequence predicts a new lipolytic enzyme with an expressed human orthologue.

**LL mRNA in Lacrimal Gland, Harderian Gland, Heart, Liver, and Pancreas**

To determine the approximate size and tissue distribution of LL mRNA, we examined LL expression in RNA blots. We isolated poly(A)^+ RNA from rabbit lacrimal gland, harderian gland, heart, liver, and pancreas; electrophoresed the RNA in denaturing agarose gels; and transferred the RNA to positively charged nylon membrane. The 3' RACE-PCR fragment of LL hybridized to a 2.1-kb band and a less intense 3.0-kb band in each of the RNA samples from harderian gland, lacrimal gland, heart, liver, and pancreas (Fig. 3A). Liver RNA hybridized relatively strongly, whereas pancreas and lacrimal gland RNA hybridized moderately, and heart and harderian gland RNA hybridized weakly to the LL probe.

We stripped the RNA blot and hybridized it with actin (Fig. 3B). Mouse α-actin hybridized to 1.5-kb rabbit α-actin and cross-hybridized to 1.9-kb rabbit β- and γ-actins, demonstrating...
the presence of intact RNA in each lane. Included for comparison is a photograph of the same RNA samples, electrophoresed in agarose gels, and stained with ethidium bromide (Fig. 3C). These poly(A)⁺ RNA samples contained ribosomal RNA carryover, allowing visualization of the 18S and 28S ribosomal bands. On the basis of the ethidium bromide staining of ribosomal bands and the LL and actin hybridization signals, the male harderian gland sample appears to contain less RNA than the female harderian gland sample.

The variable levels of actin hybridization among different tissues suggest real tissue-specific differences in β- and γ-actin mRNA levels. The actin results highlight the differences in LL mRNA expression among the rabbit tissues examined. Heart and harderian gland exhibited low levels of LL mRNA expression, but relatively high levels of actin expression. Conversely, lacrimal gland exhibited moderate expression of LL mRNA, but low expression of actin. Inclusion of RNA from both male and female rabbit lacrimal and harderian glands indicated no evidence of gender-based differential expression of LL mRNA in these glands.

To determine whether the nonoverlapping 5' and 3' LL RACE-PCR clones hybridized to mRNAs of the same size, we hybridized the 32P-labeled, 5' RACE-PCR fragment of LL to a duplicate RNA blot. We obtained the same 2.1-kb and fainter 3.0-kb band hybridizations with the same relative intensities in the duplicate blot (data not shown).

LL mRNA was expressed in all rabbit tissues examined: lacrimal gland, harderian gland, heart, liver, and pancreas. LL may be constitutively expressed.

**DISCUSSION**

**II. Expression Patterns**

Using degenerate primer PCR, we screened rabbit lacrimal glands for the expression of mRNAs containing triacylglycerol lipase sequence motifs. Our screen identified mRNAs predicted to encode a new lipase sequence, which we called LL. Although we first identified the 2.1-kb LL mRNA in the lacrimal gland, we found evidence of its expression in several rabbit tissues, implying that the putative enzyme probably performs a function in many tissues of the body.

Rabbit LL mRNA expression levels were highest in the liver and pancreas, moderate in the lacrimal gland, and lowest in the heart and harderian gland. Based on visual inspection of hybridization intensities, mRNA isolated from male and female lacrimal and harderian glands exhibited no gender-based differences in expression levels.

mRNAs encoding other lipolytic enzymes have been identified in the lacrimal glands of several species. Human lacrimal glands express PLA2 mRNA, and the corresponding protein product has been found in human lacrimal gland and tears. Female mice express mRNA for PLRP1 in lacrimal gland acinar cells, implying that the protein product is secreted in tears. The lacrimal glands of rabbits, mice, and rats also synthesize lipoprotein lipase mRNAs.

**II. Sequence Attributes**

BLAST comparisons of the predicted amino acid sequence of rabbit LL with translations of the GenBank nucleic acid data-
Historic lipase) cleave triacylglycerol to monoacylglycerol and free glycerol lipases (lipoprotein lipase, hepatic lipase, and pancreatic lipase). Several lipases and phospholipases are expressed by ocular surface epithelial cells, including meibomian glands, and by resident conjunctival and corneal epithelial cells. The putative enzymatic lipase features include the catalytic triad residues, suggesting a possible substrate and enzymatic activity for rabbit LL. Another related enzyme PS-PLA1 hydrolyzes fatty acids, and exhibit some activity toward phospholipids. PLRP2 also hydrolyzes galactolipids in vitro; however, no significant lipolytic activity has been demonstrated for PLRP1. The rabbit LL sequence predicts a new putative lipase or phospholipase.

**Possible Physiologic Functions of LL**

If the mRNA for rabbit LL is translated into protein, the resultant product would likely function as a lipolytic or lipid synthetic enzyme. Lipids in the plasma membrane of lacrimal gland cells may serve as substrates for rabbit LL. (Note that a plasma membrane association was recently reported for the human orthologue, mPA-PLA1, in cells expressing mPA-PLA1 mRNA.) If rabbit LL is secreted by the lacrimal glands, potential endogenous lipid substrates for the enzyme reside in the plasma membranes of cells in contact with the ocular tears and in the tear film lipid layer secreted by the meibomian glands. Tear film phospholipids have been suggested as a substrate for PLA2. Possible exogenous substrates for ocular tear lipases include cell surface lipids of contaminating microbes.

The putative LL enzyme could participate in lipid signaling pathways. Lipid mediators generated by hydrolysis of plasma membrane lipids or tear film lipids could provide signals to cells in contact with the ocular tear fluid, including cells of the immune system. Lipolytic enzymes or lipid mediators in the tears could contribute to appropriate or inappropriate inflammatory responses.

Endogenous lipolytic enzymes in the tears could function as antimicrobial agents, as has been suggested of human PLA2. Another possibility is that lipases in tears are necessary to metabolize lipids at the lipid-aqueous interface of the tear lipid layer. Such hypothetical enzyme activity may be necessary to generate a normal lipid composition from the raw lipid material supplied by the meibomian glands. Indeed, differences between human meibomian gland and tear film lipids have been reported. Multiple functional roles for lipolytic enzymes in tears are not mutually exclusive.

Inappropriate lipolytic enzyme expression by the lacrimal gland could have deleterious consequences for the health of the ocular surface. The ratio of hydrophobic-to-polar tear film lipids may be more important than the exact lipid composition in determining healthy lipid layer function. An imbalance of endogenous tear lipase activities could lead to a lipid layer with a compromised protective function. Such an imbalance could result from changes in the expression pattern of the lipases themselves, or changes in the expression or activity of hypothetical enzyme regulatory molecules. Ocular surface health may depend on the dynamic interactions of many different tear constituents, originating from the lacrimal glands and the meibomian glands, as well as from the cells of the conjunctiva and cornea.

**References**


