Human 12(R)-Lipoxygenase and the Mouse Ortholog
MOLECULAR CLONING, EXPRESSION, AND GENE CHROMOSOMAL ASSIGNMENT*
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Expressed sequence tag information was used to clone the full-length sequence for a new human lipoxygenase from the B cell line CCL-156. A related mouse sequence with 83% nucleotide identity to the human sequence was also cloned. The human lipoxygenase, when expressed via the baculovirus/insect cell system produced an ~80-kDa protein capable of metabolizing arachidonic acid to a product identified as 12-hydroxyeicosatetraenoic acid by mass spectrometry. Using chiral phase-high performance liquid chromatography, the product was identified as >98% 12(R)-hydroxyeicosatetraenoic acid as opposed to the S-stereoisomer formed by all other known mammalian lipoxygenases. The single copy human 12(R)-lipoxygenase gene was localized to the chromosome 17p13 region, the locus where most other lipoxygenase genes are known to reside. By reverse transcription-polymerase chain reaction, but not by Northern blot, analysis the 12(R)-lipoxygenase mRNA was detected in B cells and adult skin. However, the related mouse lipoxygenase mRNA was highly expressed in epidermis of newborn mice and to a lesser extent in adult brain cortex. By in situ hybridization the mouse lipoxygenase gene was demonstrated to be temporally and spatially regulated during embryogenesis. Expression was induced at embryonic day 15.5 in epidermis, nasal epithelium, and surface of the tongue. These results broaden the mammalian lipoxygenase family to include a 12(R)-lipoxygenase whose biological function remains to be determined.

Lipoxygenases are a family of non-heme iron-containing dioxygenases that introduce molecular oxygen stereospecifically into 1,4-cis,cis-pentadiene containing polyunsaturated fatty acids (1). These enzymes are distributed widely throughout the plant and animal kingdoms, including lower organisms like marine algae, slime molds, and coral (2, 3), but not in bacteria and yeast. Lipoxygenase metabolism leads to the biosynthesis of various bioactive lipid mediators, including leukotrienes, lipoxins, and plant signaling molecules like jasmonic acid (4, 5).

For many years the common viewpoint in the lipoxygenase field was considered to be represented by three major lipoxygenase groups in mammals designated for their positional specificity of molecular oxygen insertion onto arachidonic acid and their cell-specific expression pattern. These major lipoxygenase forms included the neutrophil 5-lipoxygenase, platelet 12-lipoxygenase, and reticulocyte 15-lipoxygenase (6, 7). Several research groups found evidence for a subdivision of the 12-lipoxygenase branch into two classes: platelet-type and leukocyte-type (6, 8–10). Besides different sites of expression, these enzymes were found to have distinctly different substrate specificities, enzyme kinetic properties, and immunoreactivity (8–11). Cloning of the two 12-lipoxygenase cDNAs indicated that these proteins were approximately 60% identical at the deduced amino acid level (12–14). In the past 2 years it has become evident through molecular cloning studies that the lipoxygenase family is much broader than originally defined. A cDNA encoding an epidermal lipoxygenase that catalyzes the formation of predominantly 12(S)-HETE1 from arachidonic acid was cloned from murine newborn skin (15, 16). Brash et al. (17) recently described the molecular cloning and expression of a second form of 15-lipoxygenase found in human skin and hair follicles, and in mice a related 8(S)-lipoxygenase cDNA was cloned (18). Beginning with expressed sequence tags in the public data bases, we describe now the characterization of a human lipoxygenase that encodes a new class of R stereospecific lipoxygenases as well as the related mouse sequence.

EXPERIMENTAL PROCEDURES

Data Base Search Methods
A file of all known animal lipoxygenases was created and each sequence used as a query for a BLASTP search against the GenBankI14 nonredundant protein data base and TBLASTN search against the expressed sequence tag data base (dbEST). Results were analyzed using BlastView, a graphical java application developed by the Computational Biology and Informatics Laboratory in the University of Pennsylvania Center for Bioinformatics.

RNA Isolation
RNA was prepared from 10⁷ cells or 100 mg tissue (newborn mouse whole body, embryonic day 13.5 (E13.5) mouse embryo, or human skin) with TRIzol reagent (1 ml; Life Technologies, Inc.).

The abbreviations used are: HETE, hydroxyeicosatetraenoic acid; E13.5, E14.5, E15.5, and E16.5, embryonic days 13.5, 14.5, 15.5, and 16.5, respectively; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription-polymerase chain reaction; RP-HPLC, reversed phase high performance liquid chromatography; HFLC, high performance liquid chromatography; HER, human embryonic kidney; GFP, green fluorescent protein; EST, expressed sequence tag; PAC, P1-derived artificial chromosome; bp, base pair(s); HPETE, hydroperoxyeicosatetraenoic acid; STS, sequence-tagged site.

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Generation of Recombinant Baculoviruses

The transfer vectors were co-transfected with BaculoGold DNA (PharMingen) into Sf9 insect cells to generate recombinant baculoviruses according to the manufacturer’s manual. Recombinant viruses were amplified twice more to obtain high titer stocks and were used to infect Sf9 cells for enzyme activity analysis.

Enzyme Activity Assay and HPLC Analysis

After 3 days of infection, Sf9 cells were collected, washed with phosphate-buffered saline and sonicated in 50 mM Tris-HCl (pH 6.8, 0.5% Tween 20) on ice for 5 s × 5 times. HEK 293 or COS-M6 cells were transfected with 10 μg of plasmid DNA and 100 μg of LipofectAMINE (Life Technologies, Inc.) and harvested 24–48 h later for enzyme activity assay or Western blot analysis. Sonicated suspension (100 μl) was incubated with 100 μM [14C]arachidonic acid or other unlabeled fatty acids at 37°C for 30 min. Stop solution (200 μl; MeCN:MeOH:H2OAc = 350:150:1) was added, and the mixture placed on ice for 5 min and centrifuged at 13,000 rpm for 15 min. The supernatant was analyzed by RP-HPLC analysis using a Hewlett-Packard 1050 system equipped with a C18 Columbus column (Phenomenex, 5 μm, 250 × 4.6 mm) with a solvent of hexane/isopropanol/acetic acid (100:1:0.1) and flow rate of 1 ml/min and by mass spectrometry.

RP-HPLC/Electrospray Mass Spectrometric Identification of Products

Aliquots of fractions collected by RP-HPLC were separated using a 1.0 mm × 15 cm Phenomenex Hypersil C18-BDS column (3 μm particle size) with an HPLC system (Applied Biosystems 140B dual syringe pump) at a flow rate of 40 μl/min with a mobile phase consisting of 70% solvent A (water with 0.005% H2OAc (pH 5.7) adjusted with NH4OH) and 30% solvent B (MeCN:MeOH:H2OAc = 85:15:0.015). The eluant was monitored by a VG Quattro II mass spectrometer equipped with a co-axial electrospray probe and triple quadrupole analyzer. For the arachidonate metabolite, the multiple reaction monitoring mode was set to m/z 327 > 214, 327 > 184 (deuterated internal standard), 319 > 208, and 319 > 179. The scanning mode was set to acquire daughter ion spectra of m/z 319 from m/z 50 to 350. Deuterated and authentic HETE standards were obtained from Cayman Chemical Co.

Immunoblot Analysis

Transfected cells were washed twice with phosphate-buffered saline and resuspended in 50 mM Tris-HCl (pH 7.5), 0.5% Tween 20, and sonicated with a microprobe 5 s × three times. The suspension was boiled with loading buffer for 3 min. Protein was subjected to electrophoresis in 10% SDS-polyacrylamide gels and electrobotted onto Hybond EC nitrocellulose and detected by a GFF monoclonal antibody (Berkley Antibody Co.) or an anti-tetrahistidine tag antibody (catalog number 34670, Qiagen).

Northern Blot Analysis

15 μg of total RNA was electrophoresed in 1% agarose/formaldehyde gels and blotted onto nylon membrane (Amersham Pharmacia Biotech). A 900-bp cDNA fragment (primers p7 + p5) was labeled with [32P]dCTP using random primers (Amersham Pharmacia Biotech). The membrane was baked at 88 °C using Bio-Dry hybridization solution (Stratagene) for 1 h and washed with 2 × SSC, 0.1% SDS at 50 °C for 15 min and 0.1 × SSC, 0.1% SDS at 60 °C for 30 min.

Southern Blot Analysis

Human genomic DNA was isolated from human blood buffy coats. 5 μg of DNA was digested with various restriction enzymes at 37 °C overnight and electrophoresed in 0.7% agarose gels, blotted onto nylon membrane (Amersham Pharmacia Biotech), and hybridized with a [32P]dCTP-labeled 88-bp PCR fragment (primers p15 and p16, Table I) contained within putative exon 6 at 68 °C.

In Situ Hybridization

A cDNA of the novel mouse lipooxygenase (as above for Northern analysis) prepared by RT-PCR and cloned directly in pCR2.1 (Invitrogen) in both orientations allowed in vitro transcription of 32P-labeled riboprobes of both sense and antisense from the T7 RNA polymerase
promoter from linearized templates. In situ hybridization was carried out using adaptations of previously described procedures (20, 21). Sections (8 μm) were hybridized, washed, dipped in photographic emulsion (Kodak NTB2), and exposed for 1 week before they were developed and fixed. Slides were photographed with Kodak slide film under dark field illumination and were scanned and assembled using Adobe Photoshop 4.0.1.

Somatic Cell Hybrid Analyses

The hybrids used in this study, MH22–6, 88H5, LS-1, HO-11, (22, 23), and JW-4 (24), have been reported previously. The rodent parents of all hybrids except 88H5 were mouse cell lines. The rodent parent of 88H5 is hamster. An 89-bp probe (p15 p16 primers) was PCR-amplified from the individual DNAs and electrophoresed in a 4% TBE (TBE = 45 mM Tris borate and 1 mM EDTA)-agarose gel.

PAC Library Hybridization and Fluorescence in Situ Hybridization Analysis

Oligonucleotides (p13 and p16; Table I) specific to the human 12(R)-lipoxygenase cDNA were hybridized to the RCPI 5 PAC library (BAC/PAC Resources, Buffalo, NY) sequentially. PACs were labeled with biotin-16 dUTP and hybridized to metaphase chromosome spreads. A digoxigenin-labeled probe for 17q-tel (Oncor) was used as control.

RESULTS

Cloning of Novel Mouse and Human Lipoxygenase cDNAs—We initiated a search of the GenBank™ and expressed sequence tag (dbEST) data bases for potential novel lipoxygenase sequences. At the time of the search (9/97), four related mouse sequences (dbEST I.D. numbers 517717, 517762, 519034, and 654412) and one human sequence (I.D. number 1352649) were identified that were related to, but distinct from, known lipoxygenase sequences. The four mouse clones were derived from a mouse day 19.5 post-coitum (E19.5) total embryo cDNA library and all overlapped, covering the region of nucleotides 1367–1893 (see below and Fig. 1). The lone human sequence derived from a tonsillar cell cDNA library that had been enriched for germinal center CD20+/IgD− B cells. This clone contained a predicted coding sequence that was highly related to the C terminus of other animal lipoxygenases and included the 3′-untranslated region to the poly(A) tail.

Using primer sequences based on the mouse EST clones and newborn total body RNA combined with 3′- and 5′-RACE techniques, we were able to amplify three overlapping fragments that covered a 2.1-kilobase full-length open reading frame encoding for a lipoxygenase of 701 amino acids (Figs. 1 and 2). 5′-RACE was used to clone the putative human ortholog from a B cell line (CCL-156; an Epstein-Barr virus-transformed B cell line from a normal human donor), which encodes a protein the same length as the mouse sequence with 86% predicted amino acid identity. These sequences were basically identical to those recently reported by Krieg et al. (25) and Boeglin et al. (26) cloned by similar procedures from mouse epidermis and human hair follicle cDNA, respectively (see Fig. 2 legend). The original human EST clone was sequenced in its entirety and was found to lack sequence corresponding to exon 7 of other known lipoxygenases.

Identification of Novel Human Lipoxygenase as an Enzyme Capable of 12(R)-HETE Formation—The full-length coding region for each of the newly isolated mouse and human lipoxygenase sequences was cloned into a mammalian cell expression vector, and the constructs were transfected into HEK 293 and COS-M6 cells. We were unable to detect any enzyme activity in our standard lipoxygenase enzyme assays in both cell types even though a control mouse platelet-type 12-lipoxygenase expressed well (data not shown). To determine whether protein was being expressed we engineered the constructs into GFP-containing vectors and examined fluorescence in living cells and protein expression by immunoblot analysis (Fig. 3). An intense perinuclear cytoplasmic fluorescence was observed for both lipoxygenases in transfected cells (Fig. 3). By Western blot analysis the predicted 110-kDa proteins were detected by use of
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H 1

H 101

H 201

H 301

H 401

H 501

H 601

Fig. 2.

Comparison of the amino acid sequences of human 12(R)-lipoxygenase and mouse ortholog. Large bold-faced residues indicate potential iron atom ligands conserved in all mammalian lipoxygenase sequences. The residues in italics and underlined indicate the 31-mnino acid region not found in any other mammalian lipoxygenase. H, human; M, mouse. The sequences have been deposited in the GenBank database, accession numbers AF059250 and AF059251. The human sequence is identical to that reported by Boeglin et al. (26). The mouse sequence (clone M) has seven nucleotide differences from the sequence reported by Krieg et al. (25), resulting in three amino acid differences and four silent changes (Val at position 9 instead of Ala; Val at position 351 instead of Met; and Ile at position 361 instead of Thr).

A GFP monoclonal antibody (Fig. 3). The GFP-lipoxygenase-expressed enzymes, like the native forms, did not possess any measurable enzyme activity.

Using a vaccinia virus-based T7 RNA polymerase system we were unable to achieve detectable lipoxygenase expression in COS-7, HEK 293, baby hamster kidney, and HeLa cells. Finally, using a baculovirus system we achieved modest expression of active human enzyme but were still unable to achieve enzymatic activity with the mouse construct, although substantial amounts of ~80-kDa protein were made (Fig. 3).

Arachidonic acid incubations of infected insect cells with the novel lipoxygenase was isolated from a human tonsillar cell cDNA library that had been enriched for germinal center B cells, human hair follicles, and skin (not shown). Since the original mouse EST clones were isolated from an E19.5 total embryonic cDNA library, we examined the expression pattern in mouse tissues. We have performed northern blot analysis in mouse thymus, tonsils, splenic B cells, and the WEHI 231 cell line as well as 15 other tissues and in 16 human tissues (human tissue blots from CLONTECH). By RT-PCR the mRNA was detected in CCL-156 B cell line for 12(R)-lipoxygenase activity. None was detectable by our standard HPLC assay using incubations with radiolabeled arachidonic acid and calcium ionophore A23187 in intact cells or in cytosolic and microsomal fractions (data not shown).

Expression Pattern of Human 12(R)-Lipoxygenase and Mouse Ortholog—Since the original human EST clone encoding this novel lipoxygenase was isolated from a human tonsillar cell cDNA library that had been enriched for germinal center B cells, we tested the CCL-156 B cell line for 12(R)-lipoxygenase activity. None was detectable by our standard HPLC assay using incubations with radiolabeled arachidonic acid and calcium ionophore A23187 in intact cells or in cytosolic and microsomal fractions (data not shown).

By chiral phase HPLC we were able to identify unequivocally the product as 12(R)-HETE (Fig. 4). Using RP-HPLC/ electrospray-mass spectrometry we were unambiguously able to identify the product as 12(R)-HETE (not shown). The peak collected at tR 12–13 min on RP-HPLC was subjected to tandem mass spectrometry analysis, and the daughter ion spectra of the molecular ion m/z 319 was the same as that of authentic standard 12-HETE, which is clearly unrelated to those of 5-, 8-, and 15-HETE (see Ref. 27). Furthermore, when 10 μl of this fraction was co-injected with 1 ng of deuterated 12-HETE, the analyte basically co-eluted with the standard using liquid chromatography/mass spectrometry/mass spectrometry (not shown).

By chiral phase HPLC we were able to identify unequivocally the product as 12(R)-HETE as opposed to the S stereochemistry products observed with all other mammalian lipoxygenases (Fig. 4). We were unable to isolate the 12(R)-HPETE precursor from the baculovirus-infected cell incubations due to the low amounts of enzymatic activity obtained.

Substrate Specificity of Human 12(R)-Lipoxygenase—We tested four additional polyunsaturated fatty acids (γ-linolenic acid (18:3), di-homo-γ-linolenic acid (20:3), eicosapentaenoic acid (20:5), and docosahexaenoic acid (22:6)), in addition to arachidonic acid (20:4), as potential substrates for the novel human 12(R)-lipoxygenase. The data are summarized in Table II. 20:3 was equally effective as 20:4 substrate, while 20:5 was metabolized at about 30% compared with 20:4. Surprisingly, 18:3 was a relatively good substrate, while the C22 compound was the poorest substrate tested. In each case, the product formed was the S9 hydroxylated compound based on mass spectrometric analysis.

Expression Pattern of Human 12(R)-Lipoxygenase and Mouse Ortholog—Since the original human EST clone encoding this novel lipoxygenase was isolated from a human tonsillar cell cDNA library that had been enriched for germinal center B cells, we tested the CCL-156 B cell line for 12(R)-lipoxygenase activity. None was detectable by our standard HPLC assay using incubations with radiolabeled arachidonic acid and calcium ionophore A23187 in intact cells or in cytosolic and microsomal fractions (data not shown).

Neither could we detect mRNA by Northern blot analysis in mouse thymus, tonsils, splenic B cells, and the WEHI 231 cell line as well as 15 other tissues and in 16 human tissues (human tissue blots from CLONTECH). By RT-PCR the mRNA was detected in CCL-156 cells, human hair follicles, and skin (not shown). Since the original mouse EST clones were isolated from an E19.5 total embryo cDNA library, we examined the expression pattern in the mouse developing embryo by Northern blot and in situ hybridization analysis. An intense hybridization signal was detected by Northern analysis in mouse thymus, tonsils, splenic B cells, and the WEHI 231 cell line as well as 15 other tissues and in 16 human tissues (human tissue blots from CLONTECH).
upper surface of the tongue, and in the nasal epithelium with the antisense probe (Fig. 6). Hybridization with a sense probe at embryonic day 16.5 revealed no distinct specific signals.

**Gene Copy Number and Chromosomal Localization**—When human genomic DNA was cleaved with 10 different restriction endonucleases, electrophoresed, and subjected to Southern blot analysis, hybridization with a putative exon 6-specific probe revealed a simple pattern indicative of a single copy 12(R)-lipoxygenase gene2 (data not shown). In order to determine the chromosomal location of the gene, we first identified two PACs (pc1156K19 and pc1080B7) by hybridization of oligonucleotides specific to the 12(R)-lipoxygenase cDNA. Each of these PACs amplified the specific 89-bp sequence-tagged site (STS) from exon 6. Fluorescence in situ hybridization was performed with each of these PACs. Analyses revealed that both PACs hybridized to chromosome 17p13 (data not shown). Next, utilizing primers specific for the 89-bp STS, we performed PCR on a panel of somatic cell hybrids that allowed regional mapping on human chromosome 17 (Fig. 7). This STS was found by PCR to be present in the hybrid MH22–6, which retains a single normal human chromosome 17. The 12(R)-lipoxygenase-specific STS was deleted in the somatic cell hybrid retaining a del(17)(pter-p13.1) (HO-11) but was present in hybrids retaining other portions of chromosome 17, including 88H5 ((17)(pter-p11.2)) and JW4 ((GM10657); del(17)(pter-p13.105)). All hybrids, except for 88H5, retain a complete long arm of chromosome 17. This STS was not present in the rodent parent lines a23 (hamster) or Cl-1D (mouse) nor was it present in LS-1, an iso-17q-retaining hybrid. These results indicate that ALOX12B maps within the 17p13.105-pter region.

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2 The human 12(R)-lipoxygenase gene has been designated ALOX12B, and other lipoxygenase gene designations have been assigned by the human gene nomenclature committee.
A human 12(R)-lipoxygenase and related mouse lipoxygenase cDNA were cloned from limited sequence data in the EST data base. The predicted coding regions for these enzymes display the typical characteristics of other mammalian lipoxygenases (6, 28, 29), including three histidines and the C-terminal isoleucine residue that would bind the putative active site non-heme iron atom (Fig. 2). The main difference for these novel lipoxygenases as compared with other sequences is the insertion of a 31-proline-rich amino acid stretch close to the N terminus.

While we were completing these studies and preparing this manuscript, two independent groups reported these

![Fig. 4](image)

**Fig. 4.** Characterization of product formed from arachidonic acid by baculovirus-expressed novel human lipoxygenase. A and B, RP-HPLC chromatograms with UV detection (A) and radioactivity counting (B). The peak eluting at 12.2 min in A (collected fraction 13, B) migrates at the retention time of 12-HETE and 8-HETE. C and D, chiral phase-HPLC chromatograms of standard compounds (approximately equal amounts of 12(R)- and 12(S)-HETE; C) and fraction 13 from A and B. The peak from the expressed enzyme eluted at the position of 12(R)-HETE (tR 11.7 min) and not at the tR of 12(S)-HETE (14.3 min).

![Fig. 5](image)

**Fig. 5.** Northern blot detection of “12(R)-lipoxygenase” mRNA in various mouse tissues and cells. RNA (15 μg) was electrophoresed, blotted, and hybridized as described under “Experimental Procedures.” Results from two different blots are depicted. An intense signal is observed in RNA obtained from 2-day-old mouse epidermis, whole body of newborn mice, and moderate hybridization in brain cortex. No positive hybridization was detected in E13.5 embryo, ovary, lung, spleen, thymus, tonsils, splenic B cells, and the WEHI 231 cell line.

![Fig. 6](image)

**Fig. 6.** Dark-field photomicrographs illustrating the temporal and spatial pattern of expression of novel mouse lipoxygenase mRNA on sagittal sections of embryos by *in situ* hybridization with 35S-labeled antisense probe. A, E12.5; B, E13.5; C, E14.5; D, E15.5; E, E16.5. F, negative control: E16.5 hybridized with sense probe. Positive signal of mRNA expression was observed only starting at E15.5, and a much enhanced signal is observed at E16.5. G, H, and I are magnified pictures of the tongue, nose, and gut skin, respectively from E16.5.

### TABLE II

| Substrate | HPLC tR (min) | Product | Molecular ion (m/z) | Major daughter ions (m/z) | Relative conversion (%) |
|-----------|---------------|---------|---------------------|--------------------------|------------------------|
| 20:4      | 13.0          | 12(R)-HETE (12h-20:4) | 319 | 179, 208 | 100  |
| 20:3      | 14.9          | 12h-20:3 | 321 | 181, 210 | 102  |
| 20:5      | 9.9           | 12h-20:5 | 317 | 179, 208 | 29   |
| 18:3      | 9.7           | 18h-18:3 | 293 | 205, 234 | 73   |
| 22:6      | 12.9          | 14h-22:6 | 345 | 153, 182 | 19   |

Substrates are: 20:4, arachidonic acid; 20:3, 8,11,14-eicosatrienoic acid; 20:5, 5,8,11,14,17-eicosapentaenoic acid; 18:3, 6,9,12-octadecatrienoic acid; 22:6, 4,7,10,13,16,19-docosahexaenoic acid. Products are the ω9 hydroxylated derivatives for each substrate.

- Determined by electrospray mass spectrometry.
- Electrospray tandem mass spectrometry for the respective molecular ions.
- 10 nmol of substrate was incubated with homogenate for 30 min at 37°C. For 20:4, the amount of product formed was 0.6 nmol.

### DISCUSSION

A human 12(R)-lipoxygenase and related mouse lipoxygenase cDNA were cloned from limited sequence data in the EST data base. The predicted coding regions for these enzymes display the typical characteristics of other mammalian lipoxygenases (6, 28, 29), including three histidines and the C-terminal isoleucine residue that would bind the putative active site non-heme iron atom (Fig. 2). The main difference for these novel lipoxygenases as compared with other sequences is the insertion of a 31-proline-rich amino acid stretch close to the N terminus. While we were completing these studies and preparing this manuscript, two independent groups reported these
sequences, cloned in similar fashion from EST information and different sources of starting RNA. Krieg et al. (25) cloned the mouse sequence, while Boeglin et al. (26) cloned the human sequence. The sequences are 86% identical at the amino acid level and are most closely related to the human 15-lipoxygenase, type 2 cloned from hair follicles, and its mouse ortholog, which encodes an 8(S)-lipoxygenase (around 50% identity) and 5-lipoxygenases of different species (about 40% identity; Fig. 8). All other mammalian lipoxygenases are less than 40% related to these new sequences.

Humans are now known to possess seven distinct lipoxygenase genes; five being functional (ALOX5, ALOX12, ALOX15, ALOX15B, ALOX12B) and two being pseudogenes (ALOX12P1, ALOX12P2) (see Refs. 17 and 30–33). Besides this newly discovered 12(R)-lipoxygenase, there is a platelet 12(S)-lipoxygenase, which is also found in epidermis (13, 34), two forms of 15(S)-lipoxygenase (17, 35), the first form found in reticulocytes, eosinophils, and trachea epithelium and the second form found in hair follicles and epidermis, and a white blood cell expressed 5(S)-lipoxygenase (36) that give rise to various HPETEs and leukotrienes. In mice, the total number of fatty acids (arachidonic acid, eicosatrienoic acid, or docosahexaenoic acid; see Table II) or may require a post-translational modification that can only be carried out in certain epidermal cells. Alterna-

We are not sure why the enzyme activity in the human 12(R)-lipoxygenase baculovirus-infected insect cells was so low.

Protein overexpression was reasonable as judged by Western blot analysis. In addition, enzyme activity could not be detected in several other commonly used systems which work well for the “classic” 5-, 12-, and 15-lipoxygenases (HEK 293 cells, baculovirus-infected insect cells, and vaccinia virus-infected HeLa cells). We and others have experienced much difficulty to get other members of the “epidermal-specific” enzymes (epidermal 12(S)-lipoxygenase, 8-lipoxygenase) to express well in HEK 293 cells and baculovirus-infected insect cells in terms of enzymatic activity (15, 18, 25, 26). We postulate that there is an epidermal-specific factor that is required to “activate” these enzymes that is not present in other types of cells. Alternatively, the epidermal lipoxygenases, including the 12(R)-lipoxygenase, may preferentially utilize an as-of-yet unidentified complex lipid substrate rather than simple polyunsaturated fatty acids (arachidonic acid, eicosatrienoic acid, or γ-linolenic acid; see Table II) or may require a post-translational modification that can only be carried out in certain epidermal cells. The low enzyme activity of the human enzyme is likely unrelated to PCR-introduced mutations in the sequence, since our sequence is identical to that of Boeglin et al. (26). The mouse enzyme, which yielded no enzyme activity under many different incubation conditions and in many expression systems, had three amino acid differences from the sequence of Krieg et al. (25). These were minor changes in noncritical regions of the protein (see Fig. 2 legend) and would not be expected to affect activity to any great extent. Similarly, this group of investigators was unable to obtain active expression of their sequence.

All human lipoxygenase genes to date, including the 12(R)-lipoxygenase gene, have been localized to the chromosome 17p.
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17p13 region, with the exception of the 5-lipoxygenase gene, which is situated on chromosome 10 (31–33, 40). Presumably, these genes arose by duplication events. The genomic structures of all the genes on chromosome 17 are very similar (14 exons and in 7–17 kilobase range). It is interesting to speculate that the 31-amino acid insertion in the lipoxygenase genes cloned in this study may have arisen by a mutation that affected splicing of exons 3 and 4 since the “extra” sequence is located at this junction. Of the genes cloned in this region to date, most have intron sizes in the range of 90–100 nucleotides in between exon 3 and 4 (i.e. the size to make a 31-amino acid insertion). If one of the splice sites was affected, the intron between exons 3 and 4 may have been incorporated into the lipoxygenase sequence. This insertion is just beyond the N-terminal β-barrel domain of lipoxygenases (residues 1 to ~125), at the start of the catalytic domain, away from the critical residues binding the non-heme iron atom (see Refs. 28 and 29). Cloning of the genomic sequence will clarify this point and may yield clues to the importance of this structural insertion for determining stereospecificity of oxygen insertion.

The human 12(R)-lipoxygenase cDNA was cloned from a B cell line, since the original EST was obtained from a germinal and may yield clues to the importance of this structural insertion for supplying B cell lines and Dr. G. Cotsarelis for the human skin sample. We thank J. A. Lawson for assistance with mass spectrometric analysis.

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