Functional Expression and Cellular Localization of a Mouse Epidermal Lipoxygenase*

(Received for publication, June 11, 1996)

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Three distinct murine lipoxygenase genes have been functionally characterized: 5-lipoxygenase (Chen, X.-S., Naumann, T. A., Kurre, U., Jenkins, N. A., Copeland, N. G., and Funk, C. D. (1995) J. Biol. Chem. 270, 17993–17999), platelet-type 12-lipoxygenase and leukocyte-type 12-lipoxygenase (Chen, X.-S., Kurre, U., Jenkins, N. A., Copeland, N. G., and Funk, C. D. (1994) J. Biol. Chem. 269, 13979–13987). Here, we describe the cloning and functional characterization of a fourth lipoxygenase gene in mice. Using a polymerase chain reaction-based approach together with partial sequence information from a genomic clone, we isolated a novel lipoxygenase cDNA from the RNA of 3–6-day-old mouse epidermis. The open reading frame predicts a 662-amino acid lipoxygenase that displays 60% identity with both murine 12-lipoxygenase isozymes and 40% identity to 5-lipoxygenase; the sequence is identical to a genomic sequence reported recently (van Dijk, K. W., Steketee, K., Havekes, L., Frants, R., and Hofker, M. (1995) Biochim. Biophys. Acta 1259, 4–8). A full-length clone was expressed in human embryonic kidney 293 cells and homogenates from disrupted cells produced 12-hydroxyeicosatetraenoic acid (12-HETE) and minor amounts of 15-HETE from arachidonic acid. Chiral phase analysis indicated that the 12-HETE is exclusively the 12S enantiomer. In situ hybridization revealed highly specific expression of epidermal lipoxygenase in differentiated keratinocytes of the epidermis and in restricted regions of the root sheath and bulb of hair follicles. High expression was also detected in conjunctiva of the eyelid and in cells of Meibomian glands. A 2.4-kilobase mRNA was detected in mouse epidermis by Northern blot analysis and its abundance was not affected by phorbol ester treatment. The epidermal lipoxygenase gene (Alox5) resides on mouse chromosome 11 closely linked with the two 12-lipoxygenase genes (Alox12p and Alox12d).

Lipoxygenases catalyze the stereospecific oxygenation of polyunsaturated fatty acids like arachidonic and linoleic acids. There are three well characterized lipoxygenases in mammals (1–3). A 5-lipoxygenase has been found in leukocytes of all mammalian species examined. Generally, blood platelets contain a 12-lipoxygenase. Rabbits and humans have a 15-lipoxygenase in reticulocytes and eosinophils, while the leukocytes of other mammals have a related enzyme, which has predominantly 12-lipoxygenase activity. The two 12-lipoxygenases have been designated “platelet-type” and “leukocyte-type” (3, 4). While the 5-, 12-, and 15-lipoxygenases have a well established occurrence in blood cells, the pattern of expression is more complex and less well characterized in skin.

Lipoxygenase activity has been detected in skin of humans, pigs, rats, guinea pigs, and mice (5–10). Nugteren and Kivits (5) found evidence for two different lipoxygenases in human and rat epidermis: a 12-lipoxygenase with ω-9 activity that converted arachidonic acid to 12-HETE and another with ω-6 activity that metabolized both arachidonic acid and linoleic acid to 15-HETE and 13-HODE, respectively. Mouse epidermis, in vitro cultured keratinocytes, and papilloma and squamous cell carcinomas express platelet-type 12-lipoxygenase mRNA, protein, and activity (11, 12). The neoplastic epidermal tumor samples displayed a constitutive overexpression of platelet-type 12-lipoxygenase (12). In addition, a phorbol ester-inducible 5-lipoxygenase activity has been detected in mouse skin (13, 14). This activity was further characterized and was found to result in stereospecific oxygenation to the 8S enantiomer (15).

Molecular cloning studies have resulted in the characterization of three distinct mouse lipoxygenase genes and their corresponding cDNAs (11, 16, 17). These include 5-lipoxygenase and the two 12-lipoxygenase isozymes, the platelet-type expressed in platelets and epidermis, and the leukocyte-type expressed in highest abundance in a subset of peritoneal macrophages. In addition to these two 12-lipoxygenase genes, both located on mouse chromosome 11, we isolated a related genomic clone originally considered to represent a pseudogene (11). van Dijk et al. (18) reported recently the complete sequence of this gene, as well as RT-PCR evidence of expression in skin, although catalytic activity of the enzyme was not ascribed. Here, we describe the isolation, functional characterization, and cellular localization of the epidermal lipoxygenase that derives from this gene.

* This work was supported in part by National Institutes of Health Grants HL53558 (to C. D. F.), AR41943 (to D. S. K.), and GM49502 (to A. R. B.) and a fellowship from the Fulbright Commission (to E. H. O.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U39200.

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1 The abbreviations used are: HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; 13-HODE, 13-hydroxyoctadecadienoic acid; TPA, 12-O-tetradecanoylphorbol-13-acetate; HEK, human embryonic kidney; RT-PCR, reverse transcriptase polymerase chain reaction; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; HPLC, high performance liquid chromatography; RP-HPLC, reversed phase-high performance liquid chromatography; SP-HPLC, straight phase-high performance liquid chromatography; kb, kilobase(s).

2 Sun, D., and Funk, C. D. (1996) J. Biol. Chem. 271, in press.

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EXPERIMENTAL PROCEDURES

Isolation of Mouse Epidermis—Dorsal/ventral skin flaps were removed from sacrificed 3–6-day-old C57BL/6 × 129 Sv mice and rinsed in phosphate-buffered saline. To separate epidermis from underlying dermal layers, the skin was placed in diethylpyrocarbonate-treated water for 30 s at 55°C, followed by a 30-s immersion in ice-cold water (19). The epidermis could then be easily peeled off in a single strip with fine forceps. Alternatively, the skin sections were placed in phosphate-buffered saline containing 10 mM DTT for 2 h, after which the epidermis was removed and rinsed free of dithiothreitol (20).

Preparation of RNA—Epidermal strips were placed in guanidinium thiocyanate, homogenized, and total RNA prepared by the method of Chomczynski and Sacchi (21). Poly(A)+ RNA was purified by oligo(dT)-cellulose spin columns (Pharmacia Biotech Inc.).

PCR Cloning of Epidermal Lipoxygenase cDNA—First strand cDNA synthesis was carried out with 1 µg of epidermal total RNA by incubation for 1 h at 37°C in 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM MgCl2, containing 800 µM dNTP, 50 pmol of random primers, 20 units of RNasin (Promega), and avian myeloblastosis reverse transcriptase (Life Technologies, Inc.) in a total volume of 25 µl. Alternatively, double-strand cDNA synthesis was carried out with the Marathon cDNA synthesis kit (Clontech) according to the manufacturer’s specifications. The initial PCR fragment was isolated with degenerate lipoxygenase primers 5′-TGGCTACCAACATGCTGGTCGG-3′ and 5′-CCAAAGTGTCACARTCNAGYTGNC-3′ using 1/10th of the starting cDNA and 20 pmol of primers and 2.5 units of Taq polymerase (Perkin-Elmer) for 35 cycles at 94°C for 45 s, 46°C for 15 s, 72°C for 1 min. The 3′ end of the cDNA was isolated by 3′-RACE using upstream primer 5′-CTGGACTGTATGCCTGGATC-3′ and an oligo(dT) adapter primer. Several primer combinations were used unsuccessfully to isolate the 5′ end. A partially characterized genomic clone A12X1 (11) that was identical to the sequence of the initial PCR clone in exon 11 was used to determine sequence at the 5′ end in exon 1. Two different primers at the 5′ end (5′-CAAGTGGTCAAGTACAGGTCCTCG-3′, initiator codon underlined; and 5′-GTTCCCTCTAGAGGATGACGCC-3′ within the 5′-untranslated region) and a primer at the stop codon (5′-CTGGAGATGTCAGACTG-3′, stop codon underlined) were used to amplify the complete coding region from double-stranded cDNA using 35 cycles at 94°C for 45 s, 56°C for 15 s, and 68°C for 2 min 30 s with a mixture of Pfu polymerase (Stratagene) and Taq polymerase (Perkin-Elmer). PCR products were purified with Wizard prep (Promega) and ligated into pCRII (Invitrogen) for sequence analysis by the deoxyxycyclase termination method.

Construction of Vectors and Transient Transfection in Human Embryonic Kidney 293 Cells—Seven separately amplified cDNA clones encoding the complete lipoxygenase were excised from pCRII with EcoRI or HindIII/NotI and cloned into either pCMV5 (22) or pCDNA3 (Invitrogen), respectively. DNA was purified by ion-exchange columns (Qiagen) and was introduced into human embryonic kidney 293 cells by calcium phosphate-mediated transfection (23). Two days later, cells were harvested and precipitated (24). Fisher model 50 sonic dissection apparatus was used on ice. Homogenates were incubated with 100 µM [14C]arachidonic acid (2 × 10³ cpm) for 30 min at room temperature in a shaking bath, and samples were extracted by the Bligh and Dyer procedure (24), modified slightly by substitution of methylene chloride for chloroform. The organic extracts were evaporated to dryness under nitrogen, redissolved in methanol, and stored at −20°C prior to HPLC analysis.

Analysis of Products—Initial screening of the product profile was carried out by RP-HPLC using a Beckman 5-µm ODS Ultrasphere column (25 × 0.46 cm) equipped with a Bio-Rad 5-µm guard column (4 × 0.4 cm), a solvent system of methanol/water/glacial acetic acid (90:10:0.01, by volume), and a flow rate of 1 ml/min. In this system, arachidonic acid has a retention time of approximately 15 min, and the HPETE products elute earlier, at retention times of 5–8 min. The column eluant was monitored on-line using a Hewlett-Packard 1040A diode array detector and a Flo-One radioactivity detector (Packard). In some cases the extracts were treated with triphenylphosphine in methanol (10 µg in 50 µl) to reduce HPETEs to HETEs prior to HPLC analysis. A solvent of methanol/water/glacial acetic acid (60:20:0.01, by volume) was used for improved resolution of the HETE products. Further characterization of the products was accomplished by SP-HPLC using an Alttech 5-µm silica column (25 × 0.46 cm) and a solvent system of hexane/isopropanol/glacial acetic acid (100:1:0.1, by volume) to separate 12-HETE, 15-HETE, and 11-HETE and their methyl esters and changed to the proportions 100:3:0:1 (by volume) to screen for synthesis of 5-HETE, 9-HETE, and 5-HETE.

In Situ Hybridization Analysis—Whole skin from CD-1 mice (Charles River Laboratories) sampled at various body locations was in situ hybridized for 24 h in a solution containing 30% formamide in 0.46 M sodium acetate, pH 7.4, dehydrated in ethanol solutions and xylenes, and embedded in paraffin. Sections (6 µm) were dried on Super Frost Plus slides (Fisher Scientific). Before hybridization, sections were dewaxed, rehydrated, and pretreated sequentially with 4% buffered paraformaldehyde, Pronase E (Protease type XIV; 110 µg/ml), and triethanolamine (0.1 M, pH 8) containing 0.25% acetic anhydride according to established procedures (19–21). A 0.42-kb SacI fragment of epidermal cloned into pBlueScript SK+ and linearized with either Sall (T3 polymerase) or XbaI (T7 polymerase) was transcribed in vitro to prepare radiolabeled sense and antisense 35S-UTP-labeled cRNA. Hybridization mixtures containing sense and antisense cRNA were applied to each half of every slide, separated by a hydrophobic barrier (PAP pen, Research Products Inc., Mount Prospect, IL). Following overnight hybridization at 50°C, sections were washed with high stringency and digested with RNase A (20 µg/ml). Sections were dehydrated in ethanol solutions containing 0.3 M ammonium acetate, dried, and dipped in Kodak NTB-2 nuclear track emulsion. After exposure for 2 weeks, the slides were developed and stained with toluidine blue. Specific hybridization was distinguished by comparing results for sense and antisense cRNAs on consecutive sections on the same slide. High magnification, brightfield illumination was used to distinguish silver grains, which have uniform size and shape, from artifacts in the emulsion or air bubbles under the coverslips. Tissues were examined at embryonic day 18.5 (term), postnatal days 6 and 12, and adulthood (1–3 mice/age, 2–6 sites/mouse).

RNA Blot and RT-PCR Analysis—Five-day-old mice were topically treated for 16–24 h on dorsal skin with either acetone (50 µl) or TPA (50 nmol/50 µl acetone). RNA preparation and cDNA synthesis were carried out as described above. Total RNA (10–20 µg) or poly(A)+ RNA (3 µg) was electrophoresed in 1% agarose/formaldehyde gels, blotted to nitrocellulose, and membranes hybridized with random prime 32P-labeled epidermal lipoxygenase cDNA (nucleotides −3 to 182) in 50% formamide, 5× SSC, 5× Denhardt’s solution, 0.5% SDS, and 100 µg/ml sonicated salmon sperm DNA for 18 h at 42°C. Membranes were washed first at low stringency at room temperature, followed by two washes of 20 min at 68°C in 0.1× SSC containing 0.1% SDS. Blots were exposed to Kodak X-Omat film at −70°C and subsequently re-probed with a human glyceroldehyde-3-phosphate dehydrogenase probe. PCR amplification of an epidermal lipoxygenase-specific 0.54-kb band was performed with sense primer 5′-AGTGACCGGCTGATGAAG-3′ and antisense primer 5′-CTCTCAGATGTCCACTG-3′ for 21–30 cycles at 94°C for 45 s, 60°C for 15 s, and 72°C for 1 min. Amplification of β-actin was also performed as an internal control. Reaction conditions were optimized for a 1.5% agarose gel, blotted to nylon membrane (Hybond-N, Amersham), and the blot hybridized with an epidermal lipoxygenase cDNA.

RESULTS

Cloning of an Epidermal Lipoxygenase cDNA—We were interested in exploring the ensemble of lipoxygenases in skin as a first step toward understanding the role of the lipoxygenase metabolites of arachidonic and linoleic acids in this tissue. A PCR-based homology approach was used to clone a cDNA encoding a novel lipoxygenase. First, we tested the exact strategy that was used previously for cloning human platelet 12-lipoxygenase cDNA (26), using primers based on the highly conserved mammalian lipoxygenase amino acid stretches WLLAK-1 (4) and WLLAK-2 (5) to prepare in vitro transcripts. 3′ RACE strategy using sequence information from clone A enabled isolation of the 3′ end. Numerous attempts to amplify the 5′ end by similar methods were not fruitful. Upon sequence analysis of clone A and the 3′ RACE product, it was noticed that the sequence was identical to exons 11, 13, and 14 from a lipoxygenase-related genomic clone (A12X1) that we had previously isolated (11). A 6.0-kb SaI fragment from A12X1 was sequenced from either end. A sequence was determined that was predicted to encode the first exon of a lipoxygenase. Additional PCR reactions

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based on this sequence information, using a combination of thermostable polymerases with proofreading activity, enabled amplification of the complete cDNA.

A 1982-nucleotide open reading frame was obtained from several independently isolated clones that would result in a predicted 662-amino-acid polypeptide, similar in size to 12- and 15-lipoxygenases, and that most closely resembles human 15-lipoxygenase (Fig. 2). The sequence contains all of the predicted iron ligands including three conserved histidines and a C-terminal isoleucine residue. The 3’-untranslated region consists of 245 bases including a polyadenylation signal, AATAAA.

**Expression of Epidermal Lipoxygenase cDNA in HEK 293 Cells**—The full-length cDNA under the control of the immediate early cytomegalovirus promoter was transfected into HEK 293 cells and lipoxygenase activity was assessed in homogenates two days later. [1-14C]Arachidonic acid was converted to a product that chromatographed with the retention time of 12-HETE and 8-HETE on RP-HPLC (Fig. 3A). The product had a UV spectrum characteristic of a mono-HETE compound with a λmax at 237 nm. No products were formed in mock transfected cells. Occasionally, a very minor peak corresponding to the position of 15-HETE was observed. SP-HPLC analysis was used to demonstrate that the S-enantiomer was virtually the exclusive 12-HETE product (>98%; Fig. 3C).

In comparison with other lipoxygenase cDNAs transfected into HEK 293 cells at the same time, it was clear that the epidermal lipoxygenase displayed very low catalytic activity (0.5–0.9% substrate conversion versus 20–25% for a human 15-lipoxygenase cDNA construct). Alteration of the incubation conditions failed to yield significantly elevated enzyme activity. Other fatty acids including linoleic acid, linolenic acid, and di-homo-y-linolenic acid were tested as potential substrates and were not converted to any appreciable extent.

**Localization of Epidermal Lipoxygenase mRNA by in Situ Hybridization**—Skin sections were prepared from a variety of mouse tissues at different stages of development and were subjected to in situ hybridization analysis for detection of the epidermal lipoxygenase mRNA. Specific hybridization with an antisense cDNA localized to discrete sites within the epidermis, hair follicles, and sebaceous glands (Fig. 4). Epidermal expression was evident in suprabasal cells located immediately above the basal cell layer, as shown in the tail skin in Fig. 4 (A and B). This restricted expression in the cells of the lower stratum spinosum was detected in skin sampled at various sites including thick skin of the footpad (Fig. 4, D and E) and thin skin of the abdomen (data not shown). In neonatal skin where all epidermal cell layers are present, specific hybridization was clearly very low or absent in basal cells and in the uppermost

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**FIG. 1. Partial restriction map of epidermal lipoxygenase cDNA isolated by PCR.** The top thick bar represents the coding region and thin line the 3’-untranslated region. The open rectangle depicts the initial PCR fragment (clone A), and closed rectangles represent subsequent cDNAs generated by PCR.

**FIG. 2.** Amino acid sequence of epidermal lipoxygenase and comparison to other related lipoxygenases. eLO, epidermal lipoxygenase; h15LO, human 15-lipoxygenase; pL12LO, porcine leukocyte-type 12-lipoxygenase; mL12LO, mouse leukocyte-type 12-lipoxygenase; mP12LO, mouse platelet-type 12-lipoxygenase. A consensus sequence is depicted underneath the five lipoxygenase sequences with (*) indicating complete identity and (.) indicating highly conserved residues with only 2 amino acids at that position. The number of amino acids in each sequence is shown at the end.

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**FIG. 3.** Partial restriction map of epidermal lipoxygenase cDNA isolated by PCR. The top thick bar represents the coding region and thin line the 3’-untranslated region. The open rectangle depicts the initial PCR fragment (clone A), and closed rectangles represent subsequent cDNAs generated by PCR.
nucleated layers of differentiated keratinocytes (upper stratum spinosum and stratum granulosum), as well as in sweat glands of the footpad (Fig. 4, D and E) and in the dermis. Epidermal lipoxygenase was also detected in the epidermis and developing hair follicles of late gestational fetal skin. A striking expression pattern was observed in the intermediate layer of fetal epidermis. Transcripts were localized to those differentiated cells within the hair pegs and were absent in the interfollicular epidermis (data not shown).

Within the hair follicles, epidermal lipoxygenase expression was detected at two distinct sites: the hair bulb and the infundibulum. Fig. 4 (A and B) shows expression in the outer layers of the hair bulb; none was detected in the matrix cells or dermal papilla. Specific hybridization in the outer root sheath of the infundibulum is shown in Fig. 4 (J and K). This includes the proximal region of the hair follicle, from the opening of the hair canal at the epidermal surface down to the opening of the sebaceous gland into the hair canal. Transcripts were absent in the distal root sheath, the region of the hair follicle between the infundibulum and hair bulb called the isthmus.

Epidermal lipoxygenase was highly expressed in the Meibomian glands in the eyelids (Fig. 4, G and H) and in the prepuce beneath the genital skin (data not shown). Specific hybridization was restricted to the cells in the outer margins of these large glands, being absent in the highly differentiated keratinocytes/sebocytes. The labeled cells appeared to be newly differentiated keratinocytes rather than proliferative undifferentiated keratinocytes (basal cells). Shown in Fig. 4 (G and H), epidermal lipoxygenase was also very highly expressed in conjunctiva, the stratified columnar mucosal lining of the eyelid. This contrasts with the expression in the immediately suprabasal keratinocytes in the keratinizing epithelium of the skin.

**RNA Blot Analysis**—RNA from the dorsal epidermis of young C57BL6 × 129 Sv mice was subjected to Northern blot analy-
Fig. 4. Localization of epidermal lipoxygenase mRNA in mouse skin by in situ hybridization analysis. Brightfield views show tissue morphology (left column; A, D, G, J). The same sections shown in darkfield (middle column; B, E, H, K) demonstrate specific hybridization with antisense cRNA. Nonspecific hybridization with sense cRNA (right column; C, F, I, L) is shown for an adjacent section on the same slide. Silver grains representing hybridization appear black in brightfield and white in darkfield illumination. A–C, tail skin at postnatal day 6 (P6) displays specific hybridization in outer cell layers of hair bulbs and in the first layer of suprabasal cells of the epidermis. D–F, thick skin of footpad at P6 showing specific hybridization in the first and second layers of suprabasal cells in epidermis, but not the outer spinous or granular cells or in sweat glands (arrowhead). G–I, eyelid at P12 showing specific hybridization in suprabasal cells of outer, keratinizing epithelium (o), in the inner mucosal epithelium (conjunctiva) (i), in hair bulbs (cut transversely; arrows), in the infundibular region of hair shafts, and in the slightly differentiated...
Murine Epidermal Lipoxygenase

Fig. 5. Expression of the epidermal lipoxygenase gene. Left panel, Northern blot analysis of epidermal RNA obtained from a 6-day-old C57BL/6 x 129 Sv mouse. Total RNA (20 μg) was loaded into a 1% agarose/formaldehyde gel, electrophoresed, blotted to nitrocellulose, and hybridized with an epidermal lipoxygenase cDNA. The arrow indicates the position of the 2.4-kb epidermal lipoxygenase transcript. Positions of 18 and 28 S ribosomal subunit bands are indicated at left. Right panel, RT-PCR analysis of epidermal lipoxygenase expression in acetone-treated (lanes 1 and 2) and TPA-treated (lanes 3 and 4) mouse skin samples. cDNA synthesis, PCR amplification, and hybridization with an epidermal lipoxygenase probe were carried out as described under “Experimental Procedures.” Lanes 1 and 3 contain equal aliquots from a 27-cycle amplification, and lanes 2 and 4 equal amounts from a 30-cycle amplification. Lane 5 was a 30-cycle negative control amplification. Standards (std) electrophoresed in parallel are represented by bars, and sizes are shown at right.

sis. Using a full-length epidermal lipoxygenase cDNA probe a transcript of 2.4 kb was detected in only one sample from a 6-day-old mouse (Fig. 5). We were unable to detect expression by this method in many other samples including RNA of skin samples from TPA-treated mice, although we could detect expression of a housekeeping gene (glyceraldehyde-3-phosphate dehydrogenase). Since it has been observed previously that TPA induces an 8S-lipoxygenase activity in mouse skin (13, 14), we assessed the ability of this compound to induce the epidermal lipoxygenase cloned here. By semi-quantitative RT-PCR, we were unable to show a significant difference of expression in TPA-treated skin versus acetone-treated skin (Fig. 5).

Organization of Epidermal Lipoxygenase Gene and Chromosomal Localization—The genomic clone A12lx1 (11) was subjected to further sequence analysis. Previously, we had obtained the sequences of exons 11, 13, and 14 (11). These sequences were identical to the sequence found in the epidermal lipoxygenase cDNA. Exon 12 was apparently absent (11). During the initial analysis of this clone, subcloned SmaI/EcoRI fragments were used for sequencing. Here, we found that one SmaI/SmaI 100-base pair segment was omitted from the initial analysis and this piece was found to contain exon 12 and was identical to the epidermal cDNA sequence. This lipoxygenase gene is unique from other lipoxygenase genes in that the boundary of exon 11/12 is shifted by one nucleotide. This gene, which was formerly referred to as Alox12ps-I5 was localized to mouse chromosome 11, closely linked with the platelet-type (Alox12p) and leukocyte-type (Alox12I) genes (11).

DISCUSSION

We have isolated and functionally characterized a cDNA encoding a fourth lipoxygenase in mice. The cDNA predicts a 662 amino acid protein that is most highly related to human 15-lipoxygenase (66% identity at amino acid level; see Fig. 2). The sequence is about 60% related to the two distinct 12-lipoxygenase isozymes in mice (platelet-type and leukocyte-type) and 40% identical to mouse 5-lipoxygenase. Interestingly, this sequence matched a partially sequenced genomic clone A12lx1 that we had isolated previously and presumed was a pseudogene. After we completed our cloning studies, van Dijk et al. (18) reported the isolation of a genomic clone related to 12- and 15-lipoxygenases using a human 15-lipoxygenase probe. The genomic locus was designated Aloxe.3 They also reported the cloning of epidermal lipoxygenase PCR clones. The sequence deduced from their genomic clone is identical to the one reported here. However, they did not report the functional activity of the lipoxygenase.

After first cloning the epidermal lipoxygenase cDNA, we surmised that this enzyme would represent the 8S-lipoxygenase that was shown to be phorbol ester-inducible in mice (13, 14). Prior experiments in our hands, using PCR amplification of cDNAs from phorbol ester-treated epidermis with degenerate, conserved lipoxygenase-specific primers, continually produced platelet-type 12-lipoxygenase, and 5-lipoxygenase clones.4 The 5-lipoxygenase was likely coming from infiltrating neutrophils, although this enzyme has been reported present in cultured keratinocytes (27). Platelet-type 12-lipoxygenase has been found previously in mouse epidermal microsomal and cytosolic homogenates (10, 11) and by immunohistochemical studies in germinai layer keratinocytes of human normal and psoriatic tissue (28).

The epidermal lipoxygenase cDNA cloned in this study when transfected into HEK 293 cells was found to generate 12S-HETE, but not 8S-HETE, from arachidonic acid (Fig. 3). C-18 fatty acids did not appear to serve as substrates. Very low enzyme activity was recovered from homogenates of transfected cells, in contrast to homogenates prepared fresh from mouse epidermis or HEK cells transfected with other cloned mammalian lipoxygenases. Many different incubation and transfection (Lipofectin versus calcium phosphate) conditions were tested, and none were found to improve the yield of product in the transfected cells. For instance, addition of calcium to the incubation medium had no effect on the activity of epidermal lipoxygenase. The possibility of PCR-derived errors in the sequence was minimized by the use of a thermostable polymerase with proofreading capabilities. Moreover, the coding sequences obtained by van Dijk et al. (18) and our group were identical, arguing against low expression due to PCR-induced mutations in the coding sequence. The novel epidermal enzyme, therefore, appears to represent a 12S-lipoxygenase, and not an 8-lipoxygenase. However, there could be alternative, better substrates for this enzyme that we have not yet found. For example, epidermis is known to contain many unique lipids, including oxygenated sphingolipid and ceramide derivatives that constitute the epidermal water permeability barrier (29–31). The precursor fatty acyl lipids could be potential substrates.

The epidermal lipoxygenase gene is closely linked to the genes for platelet-type and leukocyte-type 12-lipoxygenases (11) on mouse chromosome 11. Although all three sequences share about 60% identity, the epidermal lipoxygenase gene is approximately the same size as the leukocyte-type gene, possibly indicating a closer relationship to the latter enzyme. The platelet-type gene has some significantly larger introns. The fact that there are now three closely linked genes that can yield enzymes with 12S-lipoxygenation capability will make it even

5 Aloxe and the formerly designated locus Alox12ps-1 (11) represent the same gene locus. Since we have shown that the transcript from this gene can be functionally expressed, the designation Alox12ps-1 is incorrect and Aloxe should be employed.

4 C. D. Funk and E. H. Oliw, unpublished observations.
more difficult to examine the biology of 12-HETE.

Distinct and highly restricted patterns of expression of epidermal lipoygenase were observed in mouse skin using in situ hybridization (Fig. 4). We found that the gene was highly expressed in differentiating keratinocytes of the epidermis and hair follicles. In epidermis, the in situ hybridization data are consistent with expression in the lowest layer of spinous cells, although this was not proved by co-localization with markers of keratinocyte differentiation. This epidermal expression pattern is also consistent with results obtained for isolated keratinocytes, where the capacity for lipoygenase-dependent arachidonic acid metabolism was greater in cells having a differentiated phenotype than in those exhibiting a proliferative or basal cell phenotype (32). Our results differ, however, from those reported for immunoreactive platelet-type 12-lipoxygenase in human epidermis, which was found in basal as well as spinous cells (28). That the epidermal lipoygenase was also highly expressed in conjunctiva of the eyelid, and differentiating sebocytes of Meibomian and preputial glands, indicates that this is a gene product specific to newly differentiated keratinocytes. This is a major new finding of the in situ hybridization analyses.

Putative receptors for 12S-HETE exist on human skin Langerhans cells, keratinocytes, and the epidermal cell line SCL-II, which lends credence to an important function of this arachidonate metabolite in skin function (33–35). Perhaps the two enzymes (platelet-type 12-lipoxygenase and epidermal lipoygenase) possess unique intracellular roles (35). Perhaps the two enzymes (platelet-type 12-lipoxygenase and epidermal lipoygenase) possess unique intracellular roles (35). Perhaps the two enzymes (platelet-type 12-lipoxygenase and epidermal lipoygenase) possess unique intracellular roles (35).

Acknowledgments—We are grateful to Ginger Griffis for excellent technical assistance and to Drs. Lillian Nanney and Lloyd E. King, Jr. for insightful interpretation of in situ hybridization data.

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