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Review

The importance of the lipoxygenase-hepoxilin pathway in the mammalian epidermal barrier☆

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ABSTRACT

This review covers the background to discovery of the two key lipoxygenases (LOX) involved in epidermal barrier function, 12*R*-LOX and eLOX3, and our current views on their functioning. In the outer epidermis, their consecutive actions oxidize linoleic acid esterified in ω-hydroxy-ceramide to a hepoxilin-related derivative. The relevant background to hepoxilin and trioxilin biochemistry is briefly reviewed. We outline the evidence that linoleate in the ceramide is the natural substrate of the two LOX enzymes and our proposal for its importance in construction of the epidermal water barrier. Our hypothesis is that the oxidation promotes hydrolysis of the oxidized linoleate moiety from the ceramide. The resulting free ω-hydroxyl of the ω-hydroxyceramide is covalently bound to proteins on the surface of the corneocytes to form the corneocyte lipid envelope, a key barrier component. Understanding the role of the LOX enzymes and their hepoxilin products should provide rational approaches to ameliorative therapy for a number of the congenital ichthyoses involving compromised barrier function. This article is part of a Special Issue entitled The Important Role of Lipids in the Epidermis and their Role in the Formation and Maintenance of the Cutaneous Barrier. Guest Editors: Kenneth R. Feingold and Peter Elias.

1. Introduction

1.1. The mammalian epidermal water barrier: a short primer

One of the most important functions of the skin is the maintenance of a barrier to water vapor diffusion to the environment [1–3]. Substantial evidence demonstrates that the main determinant of the permeability properties of the skin is the lipid composition and organization in the outer layers of the epidermis, the stratum corneum [4,5]. The mammalian stratum corneum (SC) consists of flat, dead cells, called corneocytes, surrounded by a protein layer, the cornified envelope (CE), which in turn is surrounded by a monolayer of lipids, the corneocyte lipid envelope (CLE), covalently attached to the CE. The spaces between corneocytes are filled with a matrix of intercellular lipids, mainly cholesterol, free fatty acids, and ceramides, which altogether organize in layers called lamellae in mammals. The backbones of the lamellae are the ceramides, molecules formed by a sphingoid base amide-linked to a fatty acid of varying chain length [6–12]. The intercellular lamellae are thought to impede excessive evaporation through the skin, as their disruption leads to increases in transepidermal water loss (TEWL) [13,14]. Fundamental to the formation of a fully competent barrier is the need of linoleic acid, incorporated in a particular class of ceramides unique to the epidermis (EOS, esterified omega-hydroxyacyl-sphingosine) [5,15,16]. The activity of epidermal lipoxygenases (LOX) is crucial to understand the physiological process that links the need of essential fatty acids to ensure the normal formation and maintenance of the permeability barrier of the skin. Fig. 1 illustrates the main structural components of the barrier relevant to the role of LOX enzymes.

1.2. Barrier genes and ichthyosis

Genetic anomalies in the complex physiological process of epidermal barrier formation result in scaly skin diseases (ichthyoses) with medical complications like dehydration, infections, chronic blistering, and rapid-calorie loss [17–20]. Gene mutations implicated in the autosomal recessive congenital ichthyoses (ARCI) include *ALOX12B*, *ALOX3*, *CYP4F22*,

Abbreviations: ARCI, autosomal recessive congenital ichthyoses; CLE, corneocyte lipid envelope; EFA, essential fatty acid; LOX, lipoxygenase; eLOX3, epidermal lipoxygenase-3; EOS, esterified omega-hydroxyacyl-sphingosine; FATP4, fatty acid transport protein 4; H(P)ETE, hydro(pero)xyeicosatetraenoic acid; H(P)ODE, hydro(pero)xy-octadecadienoic acid; HxA, hepoxilin A; HxB, hepoxilin B; OS, omega-hydroxyacyl-sphingosine; VLFA, very long-chain fatty acid

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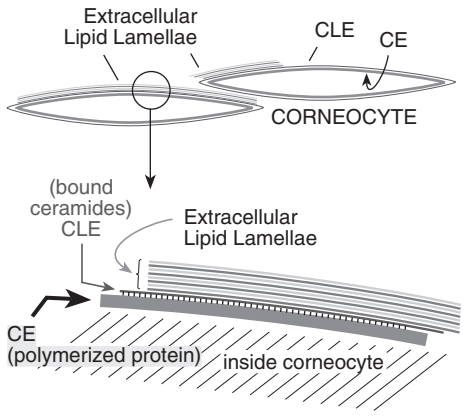

Fig. 1. Structural components of the epidermal barrier. This cartoon shows the arrangement in and around corneocytes in the barrier layer, with a close-up illustrated in the segment below. In the stratum corneum, a coat of polymerized protein, the corneocyte envelope (CE) lies just inside the periphery of the flattened corneocytes. Bonded to the CE is a monolayer of covalently-bound ceramides and fatty acids, the corneocyte lipid envelope (CLE). The CLE is considered to act as a scaffold for organization of the lamellae of extracellular lipids comprised of cholesterol, ceramides, and fatty acids.
This figure was originally published in The Journal of Biological Chemistry [87] © the American Society for Biochemistry and Molecular Biology.

ichthyin and *TGM-1* [18,21–24]. Cytochrome P450 *CYP4F22* may be responsible for synthesis of the ω-hydroxyl of ω-hydroxy-acyl ceramides. *Ichthyin* is a predicted transmembrane protein with undetermined function. *TGM-1* cross-links the proteins forming the corneocyte envelope (CE), and is also implicated through in vitro studies and a knock-in mouse mutation in catalyzing the covalent bond between ω-hydroxy-acyl ceramides and the CE, forming the CLE [25,26], (albeit with human studies showing that lamellar ichthyoses due to mutations in this isozyme have a normal CLE) [27]. The fact that inactivating mutations in all of these genes produce related skin phenotypes supports the idea that they are elements of the same physiological process.

The genetic evidence establishes that the integrity of the mammalian epidermal water barrier requires the normal functioning of 12*R*-lipoxygenase (12*R*-LOX, human gene *ALOX12B*) and epidermal lipoxygenase-3 (eLOX3, gene *ALOXE3*) [18,21,28–30]. LOX enzymes are dioxygenases that oxygenate lipids (polyunsaturated fatty acids and their esters), yet their specific role in skin barrier function is not well understood. In general terms, and taking parallels from better known LOX enzymes, their functions may be to produce oxidized lipids as signaling molecules, to induce structural changes through an enzyme-catalyzed lipid peroxidation, or rarely, to help initiate the mobilization of lipids (reviewed in Ref. [31]). Our current model suggests a function mainly related to the second of these possibilities, the induction of structural changes.

The present paper stands as an update and major revision of an earlier review [32]. Before going into specifics on our current model of the workings of 12*R*-LOX and eLOX3 in epidermal barrier function, we present overviews of the background history of the two LOX enzymes and of their essential fatty acid substrate in the skin.

2. Historical perspective on 12*R*-lipoxygenase

Mammalian 12*R*-LOX and eLOX3 remained undetected until the late 1990s. The existence of LOX enzymes in the plant kingdom was recognized over 70 years ago [33], and the more modest levels of LOX enzyme activities in animal tissues were first identified in the mid-1970s. That work established the existence of a 12*S*-LOX in blood platelets [34,35], a 5*S*-LOX in leukocytes (catalyzing the first steps in leukotriene biosynthesis) [36], and a 15*S*-LOX in reticulocytes [37]. Closer examination over the years revealed the expression of these enzymes in many other mammalian tissues [38].

The "*S*" in 12*S*-, 5*S*- and 15*S*-LOX enzymes refers to the mirror image form of the fatty acid hydroperoxide product. "*R*" chirality LOX enzymes were unknown. Then in the mid-1980s enzyme activities in invertebrate animals (corals, starfish, sea urchins) were characterized that synthesized "*R*" chirality fatty acid hydroperoxides [39]. In the mid-1990s, purification of one of these invertebrate *R*-LOX proteins and its molecular cloning revealed a close sequence homology to the plant and animal *S*-LOX enzymes [40]. Differences in substrate binding were proposed that could explain how structurally related enzymes can form products of one mirror image form or the other [40,41].

The first indication of a role for oxidized polyunsaturated fatty acids in skin biology came in the mid-1970s when an accumulation of 12-HETE (12-hydroxyeicosatetraenoic acid, i.e. 12-hydroxy-arachidonic acid) and free arachidonic acid was discovered in the involved epidermis of psoriasis [42]. At the time, it was presumed that the 12-HETE arose from the activity of 12*S*-LOX (subsequently identified in the germinal layer cells of the mammalian epidermis [43]). A decade later the psoriatic product was shown to be mainly 12*R*-HETE [44]. It took another decade of research before the "culprit" enzyme in this case was identified as the uncommon type of *R*-lipoxygenase, 12*R*-LOX, the only *R*-LOX in mammalian tissues [45,46].

Historically the enzymatic oxidation of arachidonic acid in mammalian biology shows a strong association with the biosynthesis of pro-inflammatory lipid mediators [47], and this remains a focus of eicosanoids in skin inflammation and immunity [48]. Synthesis of the prostaglandin products of the cyclooxygenase pathway is inhibited by NSAIDs, and the leukotriene products of 5-LOX have their pro-inflammatory actions blocked by receptor antagonists such as Singulair® and Accolate® [47]. Initially therefore, it was natural to equate the synthesis of 12*R*-HETE in psoriasis with the inflammation in the epidermis. Yet 12*R*-HETE itself showed only modest pro-inflammatory activity [49]. From a current perspective, the abundance of 12*R*-HETE in psoriasis may reflect merely the co-occurrence of free arachidonic acid (not present normally, but a facet of the inflammation in psoriasis) with an over-abundance of the granular cells of the upper epidermis, the natural site of 12*R*-LOX expression in healthy skin. Having the arachidonic acid available as substrate together with high expression of 12*R*-LOX may account for the excess of 12*R*-HETE in psoriatic epidermis. In fact in normal epidermis, there is only one definitive account of the synthesis of 12*R*-HETE, barely detectable as a product of [^{14}C]arachidonic acid metabolism in human hair roots [50].

In primary cultures of human keratinocytes there is no detectable 12*R*-LOX activity (they produce only traces of 15-HETE [51,52]). The absence of 12*R*-LOX is likely attributable to its association with the late stages of keratinocyte differentiation, a condition that requires special long-term treatments to mimic in culture [53], and not studied so far with regard to LOX enzyme activities. (A prominent 12-LOX activity was detected in normal human epidermis, but the *R*/*S* chirality of the 12-HETE was not determined [54]). Despite the hard to detect activity, immunohistochemical evidence clearly defines the occurrence of 12*R*-LOX (and eLOX3) in the outermost cells of the stratum granulosum in normal mammalian epidermis [29,30]. We also have evidence from in situ analysis in human skin that 12*R*-LOX mRNA expression occurs in the outermost epidermal cells that retain a nucleus (Fig. 2). Although 12*R*-LOX expression is not entirely skin-specific, no symptomatology or phenotype is reported in relation to the limited expression in other tissues. While mRNA transcripts for 12*R*-LOX are detectable by RT-PCR in several other tissues (and similarly for eLOX3) [55,56], the only instance confirmed so far with catalytically active enzyme is a weak activity of 12*R*-LOX in human tonsils [57].

The role of 12*R*-LOX in healthy skin is quite different from the earlier implications of its appearance in psoriasis, and we now know that the natural substrate of 12*R*-LOX is a linoleate ester rather than arachidonic acid. As firmly established from analysis of the gene inactivation in humans and mice, the biological role of 12*R*-LOX is helping to seal the permeability