Review Article

The Lipoxygenases: a mini review

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An International Conference on Lipoxygenases, organized by the authors, was held in Malta on May 21-24, 1997. This article reviews the field in general, involving to some extent the topics discussed during that conference. The proceedings of that conference will comprise a series of review articles covering specific aspects of the field. Publication is expected early in 1998 by Plenum Press.

Lipoxygenases (abbreviated LOX or LO) are a group of enzymes derived from a multi-gene family, identified mostly in mammalian systems, but also occur in plants. They incorporate molecular oxygen into an all *cis*-methylene-interrupted polyunsaturated fatty acid to produce a hydroperoxide with a conjugated Z,E-diene structure (Hamberg, 1971). The location of entry of oxygen into the fatty acid depends on the type of lipoxygenase; for example, 5-LO incorporates oxygen at carbon atom 5 in the fatty acid, 12-LO at the 12 position and 15-LO at the 15 position (Yamamoto, 1992). In fact, the various LO proteins have been named according to the point of entry of the molecule of oxygen into the fatty acid. The immediate product from the LO reaction is a hydroperoxide (HPETE) of distinct optical activity (*S*-configuration) (Hamberg, 1971; Nugteren, 1975; Yamamoto, 1991). It is further transformed by other enzymes into a variety of biologically active families of products, including the leukotrienes (or LTs formed from 5*S*-HPETE) (Samuelsson et al, 1987), lipoxins (or LXs formed from 5*S*- and 15*S*-HPETE) (Serhan and Romano, 1995) and hepoxilins (Hx formed from 12*S*-HPETE) (Pace-Asciak, 1994). The various LO and their products have been implicated in a variety of diseases including hypersensitivity reactions and asthma (5-LO) to atherosclerosis (15-LO), diabetes (12-LO), obesity (8-LO), and cancer (12-LO)(vide infra).

Three mammalian LO have been isolated, highly purified and their cDNAs cloned (Yamamoto, 1992; Funk, 1996). They are derived from reticulocytes (15-LO) (Kuhn et al, 1990; Sloane et al, 1990), platelets (12-LO) (Hada et al, 1991) and neutrophils and basophilic leukaemic cells (5-LO) (Furukawa et al, 1984). There are some distinguishing features in these enzymes. All contain a catalytic non-heme iron and a non-catalytic sulphur atom. Functionally, they can be distinguished by their substrate specificities; human platelet 12-LO and basophilic leukaemia cell 5-LO are highly specific for arachidonic acid (AA or ω6-C20:4) and eicosapentaenoic acid (EPA or ω3-C20:5) as substrates, while the porcine 12-LO has a broad spectrum of substrates ranging from ω6 and ω3-C18:2 and :3 to the C20 and C22 series of polyunsaturates. The rabbit reticulocyte 15-LO works best with the C18:2, :3 and C20:3 series (9, Hada et al, 1991; Yokoyama et al, 1986; Takahashi et al, 1988). Recent structural studies have shown great homology between the 15- and 12-LO. In fact site directed mutagenesis of the native 15-LO to replace the residues isoleucine (417) and methionine (418) with the smaller residue, valine, transformed 15-LO activity to form 12*S*-HPETE by the mutant, a product of the 12-LO (Sloane et al, 1991. Additional recent evidence indicated that the amino acid 353 appears to be the primary determinant of product specificity (Borngraber and Kuhn, 1997). Thus, if aa353 is occupied by a small amino acid such as valine, 12-oxygenation is preferred and aa417 and aa418 do not play a role; while if aa353 is occupied by a bulky amino acid such as phenylalanine, then aa417 and aa418 become important for determining 12-or 15-oxygenation. This reflects the extent of penetration of the substrate into the active pocket of the enzyme. Some evidence of this diversity may, in fact, also be evident in the native enzyme as the porcine 12-LO forms a small amount of 15*S*-HPETE in addition to the expected 12*S*-HPETE. Platelet 12-LO is devoid of this activity and forms exclusively 12*S*-HPETE. 5- and 15-LO appear to be multifunctional as their hydroperoxide product is further transformed to an epoxide i.e. 5,6- from 5-HPETE and 14,15- from 15-HPETE (Yamamoto,1991).

Important information on the role of proteins in mammalian physiology and patho-physiology can theoretically be obtained from animals incapable of expressing that protein through gene disruption techniques. Funk et al have bred mice made defective of a specific single LO gene (see review by Funk in the Malta LOX proceedings). They have generated animals deficient in either of the three LO (Chen et al, 1994; Chen et al, 1995; Copeland et al, 1993). Each LO-deficient mouse lacked the ability to form the HPETE specific to that LO. Results have indicated that although LO-gene

disruption did not produce gross abnormalities and hamper the animals' fertility, some interesting insights into the importance of LO and LO products have been obtained.

It is well known that LTs, historically known as SRS-A (slow reacting substance of anaphylaxis) are mediators of hypersensitivity reactions causing potent bronchoconstriction of the human airways (Dahlen et al, 1981; Samuelsson et al, 1980; Samuelsson, 1983). In fact the cysteinyl-LTs contract the isolated airways preparation about 1000-fold greater than that caused by histamine (Dahlen et al, 1980). Considerable interest has therefore been placed by the pharmaceutical industry to produce selective inhibitors of the biosynthesis or action of these compounds. LTB_4, a dihydroxy metabolite formed through the 5-LO pathway, is a potent mediator of inflammation with chemotactic and chemokinetic properties, able to recruit inflammatory cells to the site of injury (Samuelsson et al, 1980; Samuelsson, 1983; Borgeat et al, 1979; Sameulsson et al, 1979). The involvement of the cysteinyl-LTs in asthma is well established, as these compounds are potent smooth muscle constrictors, cause mucus hypersecretion, decrease mucus transport and contribute to the onset of inflammation by causing plasma extravasation and recruiting inflammatory cells (Hay et al, 1995; Dahlen et al, 1983).

5-LO disruption has led to the finding that the knock-out mice were unresponsive to aerosol antigen challenge whereas the wildtype mice developed markedly enhanced cholinergic responsiveness of similar extent to that seen in normal asthmatics (See Funk, proceedings of the Malta LOX conference). Pronounced eosinophilia (70-80% of the total bronchoalveolar lavage) was observed in wildtype mice, whereas eosinophils accounted for a small proportion of the bronchoalveolar cells in the knockout mice. This is the first evidence of the result of a functional deficiency in LT production (through the 5-LO pathway). Of significant interest is the effect of 5-LO deficiency in protection against PAF-induced shock. PAF causes a dose-dependent increase in mortality in wildtype mice, while 5-LO deficient mice show great resilience in this shock model (Chen et al, 1994). This is an important demonstration of the involvement of 5-LO products in shock and the potential rescue by inhibiting the generation of these products. Although this was achieved through LO-deletion, it paves the way for the development of selective 5-LO inhibitors for the pharmacological manipulation of 5-LO. PAF causes the lowering of arterial blood pressure leading to shock and mortality in the wildtype mouse. This is overcome in the 5-LO knockout mice after an intial drop in blood pressure resulting in survival of these mice. This may be due to the known effects of LTs in causing plasma microvascular leakage and bronchoconstriction, vide supra. These studies reaffirm rather conclusively the pathophysiological role of the LTs in asthma and related inflammatory states.

Within the 12-LO pathway, two major pathways have been identified. The extent of direction of the initial 12-LOX product, 12-HPETE, is fine tuned by the action of glutathione peroxidase, an abundant enzyme in the cell cytosol. One pathway involves the reduction of 12*S*-HPETE to 12*S*-HETE by glutathione peroxidase (Bryant and Bailey, 1980), while the second pathway involves the isomerization of 12*S*-HPETE by hepoxilin synthase into the hepoxilins (Pace-Asciak et al, 1983; Pace-Asciak and Martin, 1984; Pace-Asciak, 1984; Reynaud et al, 1994). 12*S*-HETE has been shown to potently promote the attachment of tumour cells to vascular endothelium, implicating a role for this compound in tumour metastasis (Raz et al, 1993; Liu et al, 1994; Tang et al, 1993; Tang et al, 1995). In this process, 12*S*-HETE inhibits cell apoptosis, and promotes tumour cell survival, a condition that favours tumour cell attachment and metastasis. In fact inhibitors of 12-LO appear to favour tumour cell apoptosis and therefore may find application in cancer therapy (see review by Honn in the Malta LOX proceedings). Hepoxilins, the other half of the equation, have been identified as intrinsic factors which regulate platelet cell volume (Margalit et al, 1993) and which play a role in ion (Ca^{2+} and K^{+}) fluxes in the cell (see review in Pace-Asciak, 1994). They have also been implicated in insulin secretion and brain function. Use of leukocyte-type 12-LO knockout mice have not been as successful in identifying a phenotype as has been stated above with the disruption of the 5-LO gene (Sun and Funk, 1996). One should remember that with 12-LO, two types of enzyme have been described, i.e. the platelet-type and the leukocyte-type. Leukocyte-type 12-LO knockouts appear normal, and their macrophages appear to still be able to form about 20% of 15-LO products (normally these are side products of this type of 12-LO) and an enhanced production of 5-LO products (probably through a redirection of fatty acid substrate). The leukocyte-type 12-LO knockout mice appear to have normal behaviour in tumour metastatic potential (Sun and Funk, 1996), while other parameters (involved in hepoxilin action) have not yet been investigated. It is possible that the other phenotypes may become apparent when the platelet-type 12-LO gene is deleted. Currently little information is available on these knockouts, but platelet-type 12-LO knockout mice (see review by Funk in the Malta LOX proceedings) also appear to show similar platelet and megakaryocyte numbers as wildtype mice, and to show similar adherence to extracellular matrix proteins. However, platelets from the deficient mice show enhanced responsiveness to ADP (3 - 4 times) in both in vitro and in vivo thromboembolism studies induced by ADP administration. It is not known which particular 12-LO product may be responsible for this effect, either 12-HETE or any of the hepoxilins. It is attractive to link the known inhibitory effects of 12-HPETE or 12-HETE on platelet thromboxane production and aggregation to the hyperresponsiveness of platelets from 12-LO knockout mice. These experiments would support the involvement of 12-LO in platelet aggregation and tumour metastasis, which requires tumour cell docking at sites on the vascular endothelium rich in platelet clumps. Recent

studies with 12*S*-HPETE have suggested that at micromolar concentrations, 12*S*-HPETE (but not 12*S*-HETE) causes a potentiation of aggregation induced by subaggregating concentrations of arachidonic acid (see review by Lagarde in the Malta LOX proceedings). Hence platelets appear to be 'primed' to aggregate by physiological concentrations of 12*S*-HPETE.

15-LO has been implicated in lipoprotein modification through oxidation of HDL. Together with 5-LO, 15-LO is involved in the synthesis of lipoxins (LX), trihydroxytetraene-containing derivatives of arachidonic acid (Samuelsson et al, 1987; Serhan and Drazen, 1997; Serhan, 1989) LX are formed within the vascular lumen through the interaction of platelets and leukocytes and at mucosal surfaces by leukocyte-epithelial interactions. LX are vasoactive hormones displaying selective actions on human leukocytes including inhibition of neutrophil chemotaxis, neutrophil transmigration through epithelial and endothelial cells. They are consequently involved in multicellular responses such as inflammation, atherosclerosis and thrombosis (see review by Serhan in the Malta LOX proceedings). But to date, no information is available on 15-LO knockouts, or implications of specific phenotypes relating to inhibition of LX formation. But this is certainly an area that we will be hearing about in the near future.

Other LOs are known to exist on the basis of the isolation of the corresponding HETEs although little is known regarding the purification or properties of these LOs. Of the products formed by these LOs, 8-HETE has been shown to have interesting biological actions relating to specific binding to the nuclear receptor, PPARα (Evans, 1997). Peroxisome proliferator activating receptors (PPARs) are members of the steroid/thyroid/retinoid nuclear receptor superfamily of transcriptional factors implicated in the control of lipid metabolism (Lemberger et al, 1996; Devchand et al, 1996). Three receptor subtypes have been identified, α, β and γ (see review in Lemberger et al, 1996). PPARs appear to play a role in the regulation of lipid metabolism (fatty acid oxidation) but recent studies have indicated that these receptors may also be involved in cell growth, differentiation and death and therefore involved in tumour progression. The subtype appears to be upregulated in intestinal epithelial cells in some human polymorphs (Samid et al, 1997; Brockman et al, 1997), but the biological significance of this finding is not yet understood. Prostaglandins of the J_2 type, i.e. 15-deoxy-Δ-12,14-prostaglandin J_2 appear to have the highest activity of all ligands tested to bind to and activate PPARγ promoting differentiation of pre-adipocytes into mature triglyceride forming fat cells (Forman et al, 1995), while 8S-HETE appears to be selective for PPARα (Evans, 1997). The latter appears to regulate fatty acid oxidation. Hence the LOs and their products, may be involved in the regulation of obesity and diabetes as well as in cancer therapy through their actions on PPARs. 8-HETE, among many eicosanoids tested, has also been implicated in oocyte maturation (Meijer et al, 1986).

LO enzymes appear to be derived from separate genes, each consisting of 14 exons located on chromosome 11 (Funk, 1996; Funk, 1993), although the 5-LO appears distinct being located on chromosome 11 in man and central chromosome 6 in the mouse (Chen et al, 1994; Funk et al, 1992). Some LO have a greater degree of homology than others as shown in the following phylogenetic scheme which depicts the various mammalian LO types and the relationship to each other. The x-ray crystal structure of 15-LO from soybean has been reported (Boyington et al, 1994).

LOs have also been proposed in plants and seeds. An 8R-LO has been isolated from the marine coral *P. homomalla* and cloned (Brash et al, 1996), and a complex series of LO products (collectively called oxylipins) have been isolated from the marine red algae and proposed to have osmoregulatory functions (see review by Gerwick in the Malta LOX proceedings). In seeds, the activation of an LO is proposed to be involved in the germination process (Feussner and Kuhn, 1995). 15-LO products analogous to the hepoxilins derived from 12-LO, have been isolated from roots of the common edible garlic (Pace-Asciak et al, unpublished) but its significance is not well understood at this time.

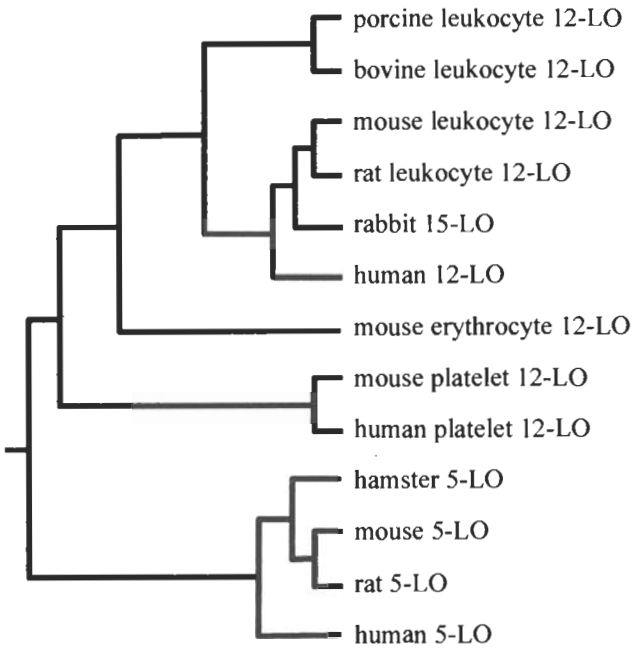

Figure 1. Phylogenetic tree of the LO gene family (courtesy of Dr. Colin Funk, University of Pennsylvania)

Future directions in this field will certainly involve the knockout mouse models, possible involving multiple knockouts, to better understand the physiological and pathophysiological role(s) of specific LOs and their immediate products. Studies with 5-LO knockouts have already provided good evidence of the implication of LTs in asthma. Cancer, obesity, diabetes and cardiovascular disease may be obvious phenotypes to investigate future gene disruption studies.

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