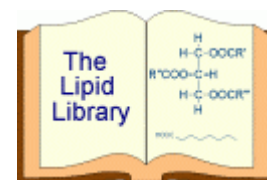


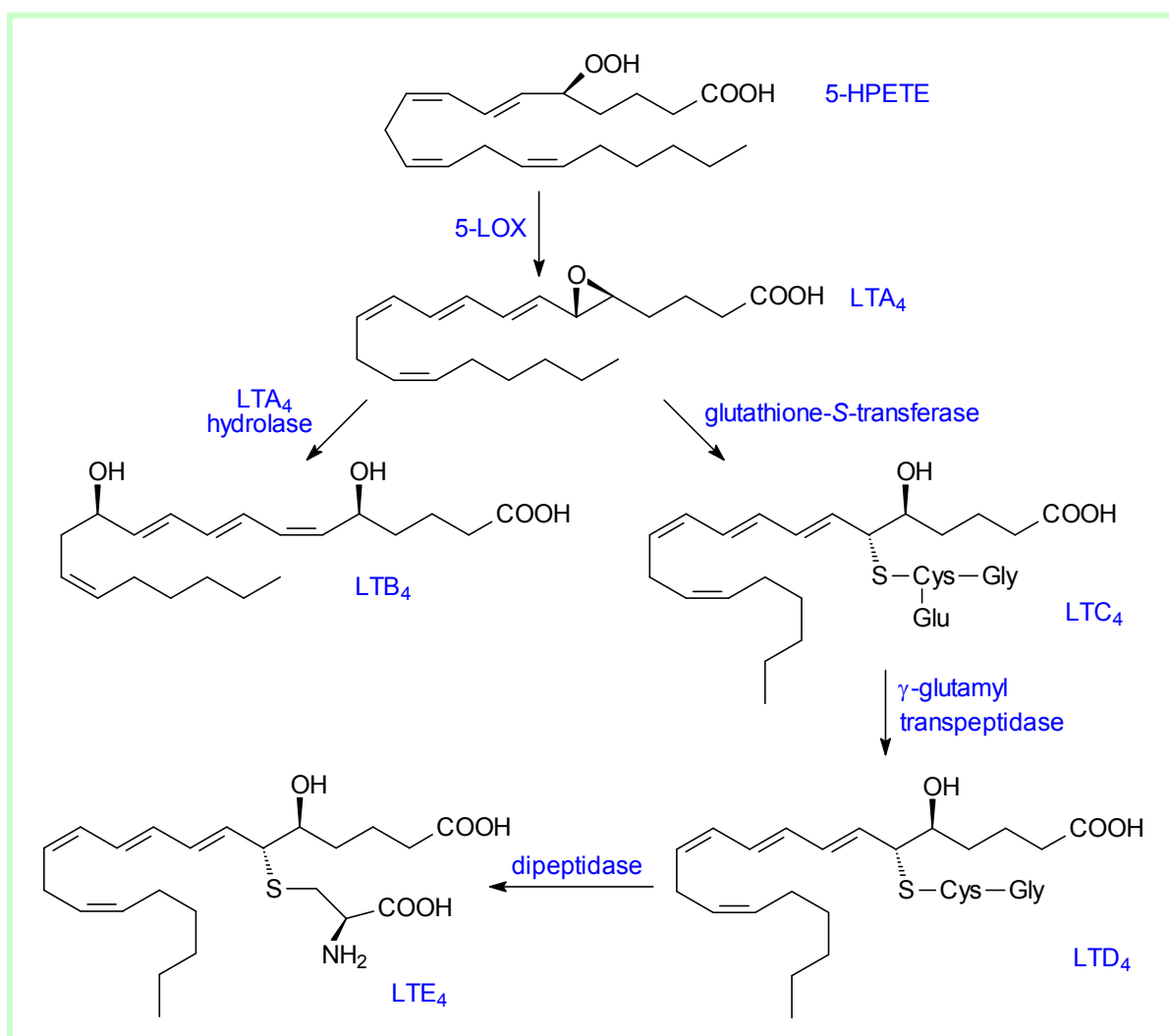
# LEUKOTRIENES AND LIPOXINS

## Chemistry and Biology



### 1. Leukotrienes

The term 'leukotriene' was coined because these important eicosanoids were first discovered in the white blood cells derived from bone marrow, i.e. the leukocytes, and they have three double bonds in conjugation (though they have four in total), resulting in specific absorbance peaks in their UV spectra (at 270, 280 and 290 nm). They are known to exhibit a wide range of biological activities, most of which involve some form of signalling function akin to that of short-lived paracrine reagents. The structures and basic mechanism for biosynthesis are illustrated below.



The biosynthetic precursor of the leukotrienes is arachidonic acid released from phospholipids, and this is acted upon by a series of enzymes, each of which has a high stereospecificity, starting with 5-lipoxygenase (5-LOX) and generation of 5S-hydroperoxy-6*t*,8*c*,11*c*,14*c*-eicosatetraenoic acid (5HPETE) by the incorporation of one molecule of oxygen at the C-5 position (see the **Introduction** to this series of documents for a discussion of the properties of lipoxygenases in general). In contrast to other lipoxygenases, 5-LOX requires the presence of 5-lipoxygenase

activating protein (FLAP), located in the nuclear envelope, to function properly. It is believed that FLAP exists as a trimer, which contains a binding pocket for arachidonic acid, from which the latter can interact with the 5-LOX catalytic domain and enable transfer to the active site. Although its structure has not been fully determined, 5-LOX is believed to contain a catalytic domain and an *N*-terminal domain, which binds calcium and zwitterionic phosphatidylcholine in membranes but not cationic phospholipids. These are essential for its activity. In addition, the activity of the enzyme is regulated by phosphorylation at three serine residues by specific kinases.

In humans, 5-LOX is expressed mainly in leukocytes, dendritic cells and in foam cells of atherosclerotic tissue (in other cells synthesis is blocked by DNA methylation). In resting cells, 5-LOX occurs either in the cytosol or in the nucleus as a soluble enzyme, depending on the cell type. It is then believed to co-migrate with phospholipase  $A_2$  to the nucleus where the latter liberates arachidonic acid from phospholipids for transfer by FLAP to 5-LOX for metabolism. Little leukotriene synthesis occurs in resting cells, but it is stimulated by cellular events that raise the level of calcium ions. It has also become apparent that some of these transformations can occur in one cell type (donor cell) before the intermediate is passed to a second cell type (acceptor cell) to complete the conversion into the biologically active mediator. Mechanisms must exist to transport the eicosanoid intermediate between cells and across phospholipid membrane barriers. For example, some cells lack the 5-lipoxygenase, but are able to synthesise leukotrienes by this cooperative process known as trans-cellular biosynthesis.

5-HPETE can be released as such and reduced to 5*S*-hydroxy-eicosatetraenoic acid (5-HETE). However, 5-LOX has a dual function in leukotriene synthesis as it also catalyses the second step illustrated above, i.e. the transformation of 5-HPETE into 5,6-epoxy-7*t*,9*t*,11*c*,14*c*-eicosatetraenoic acid or leukotriene  $A_4$  ( $LTA_4$ ), which is the first of the leukotrienes. The 3- and 5-series leukotrienes have eicosatrienoic and eicosapentaenoic acids, respectively, as the precursors.  $LTA_4$  is highly unstable with a half-life of only ten seconds at pH 7.4 *in vitro*, although it is stabilized to some extent by binding to albumin or by inclusion in phospholipid liposomes. However, if it is not metabolized quickly, it can be transformed by non-enzymic hydrolysis of the epoxide ring into a variety of dihydroxy acids with relatively little biological activity (all four stereoisomers of  $LTB_4$ ).

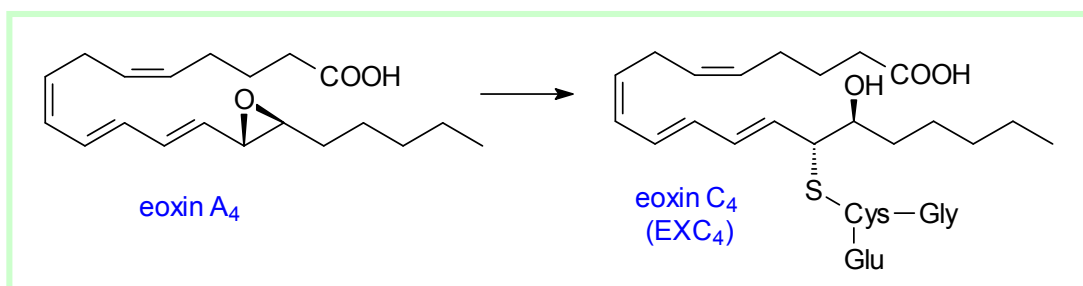
The enzymic reactions leading to the dihydroxy acid  $LTB_4$  and the peptide-leukotrienes, especially  $LTC_4$ , are much more important from a biological standpoint and their synthesis is controlled by the location of the enzymes for each product in specific types of cells in humans.  $LTA_4$  synthesised in erythrocytes is the precursor for leukotriene  $LTB_4$  or 5*S*,12*R*-dihydroxy-6*c*,8*t*,10*t*,14*c*-eicosatetraenoic acid, which is synthesised by the action of the enzyme  $LTA_4$  hydrolase ( $LTB_4$  synthase, a zinc-dependant metallo-protein). This has a dual activity as an aminopeptidase and is located mainly in neutrophils. Unlike most other enzymes involved in the 'leukotriene cascade', it is present in the cytosol of the cell, so there must be some mechanism to ensure that it is close to the nuclear membrane where the other steps in the process occur.

Alternatively,  $LTA_4$  generated externally is acted upon by  $LTC_4$  synthase or glutathione-*S*-transferase, which is found on the nuclear envelope of cells and adds the tripeptide glutathione ( $\gamma$ -glutamyl-cysteinyl glycine) to carbon 6 to yield peptido-leukotriene  $C_4$  ( $LTC_4$ , a 'cysteinyl leukotriene'). This enzyme is found mainly in mast cells and eosinophils, although it has also been detected in platelets and epithelial cells. Removal of glutamic acid by a  $\gamma$ -glutamyl transpeptidase gives leukotriene  $D_4$ , and further hydrolysis by a dipeptidase gives leukotriene  $E_4$  in which cysteine only is linked to the eicosanoid moiety.  $LTA_4$  can also function as a precursor of the lipoxins.

## 2. Eoxins

Recently, novel eicosanoids related to the cysteinyl-leukotrienes were characterized as products of the 12/15-lipoxygenase (15-LOX-1) human eosinophils and mast cells. The primary product of the

lipoxygenase, 15-HPETE is believed to react with the enzyme further to produce the 14,15-epoxide, designated eoxin A<sub>4</sub>, and then by analogy with leukotriene biosynthesis this in turn reacts with glutathione to produce eoxin C<sub>4</sub>, and thence eoxin D<sub>4</sub> (linked to Cys-Gly) and eoxin E<sub>4</sub> (linked to Cys only).

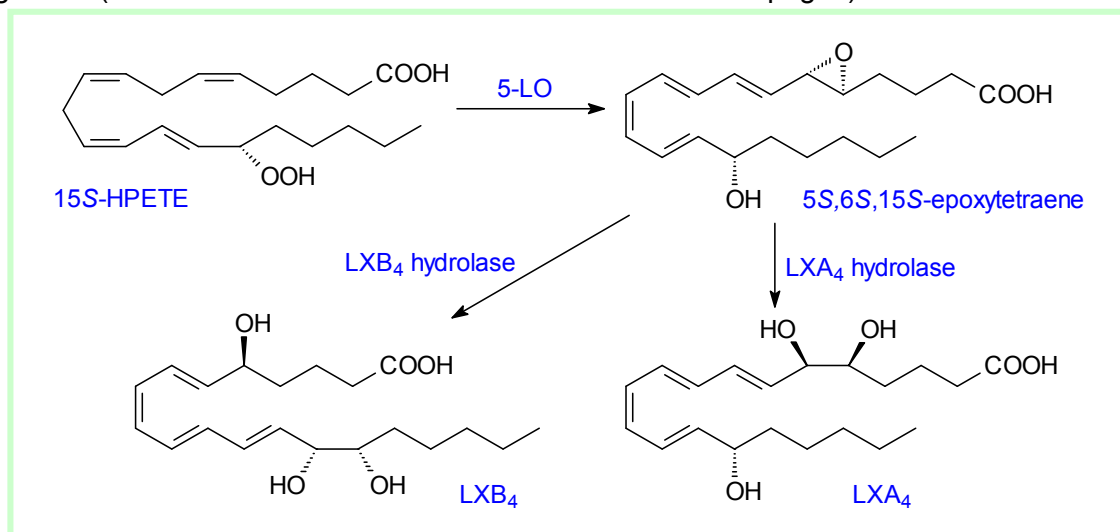


Like the cysteinyl-leukotrienes, the eoxins are potent pro-inflammatory agents.

### 3. Lipoxins and Related Compounds

**Lipoxins** are trihydroxy-eicosatetraenoic acids, derived from arachidonic acid with the four double bonds in conjugation, which were the first lipid mediators to be discovered that were involved in the resolution phase of inflammation (like the resolvins). These molecules have structural similarities to the leukotrienes and appear to have some complementary biological activities. They are also formed by trans-cellular pathways.

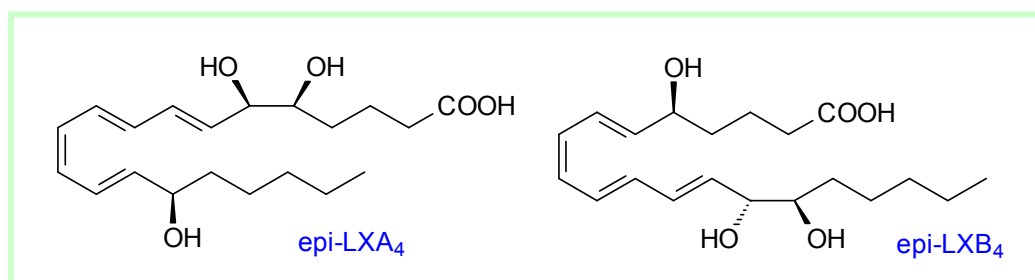
There are at least three routes to the biosynthesis of lipoxins that differ among cell types. However, a common feature is the insertion of molecular oxygen at two sites in arachidonic acid by distinct lipoxygenases. For example as illustrated for the biosynthesis of the lipoxins designated A<sub>4</sub> (LXA<sub>4</sub>) and B<sub>4</sub> (LXB<sub>4</sub>) by one of the recognized mechanisms (in airway epithelial cells and monocytes), the first step is the formation of 15S-hydroperoxy-5c,8c,11c,13t-eicosatetraenoic acid by a 15-lipoxygenase (15-LO - see the **Introduction** to this series of web pages).



This or the reduced form 15S-HETE is then acted upon by a 5-lipoxygenase to form first an epoxy intermediate, i.e. 5S,6S-epoxy-15S-hydroxy-E<sub>4</sub> and then, depending on the cell type, by specific hydrolases to form either 5S,6R,15S-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid (LXA<sub>4</sub>), or to 5S,14R,15S-trihydroxy-6,10,12-*trans*-8-*cis*-eicosatetraenoic acid (LXB<sub>4</sub>). In both products, the stereochemistry of the carbon 15S hydroxyl group is retained. In a second mechanism in which leukocyte-platelet interactions are involved via the same epoxy intermediate, the initial step is the action of a 5-lipoxygenase (to form leukotriene A<sub>4</sub>), before the reaction of the 15-lipoxygenase

(platelets are not able to produce lipoxins on their own). The precursor 15-HETE is found esterified to phosphatidylinositol, and may be a storage form in the membranes of inflammatory cells, released on stimulation. Overall, these reactions also reduce leukotriene formation.

An important third mechanism has recently been discovered that produces lipoxins of different stereochemistry, i.e. the **epi-lipoxins**, sometimes termed the aspirin-triggered lipoxins ('ATL'), as the reaction is initiated by aspirin and requires the cyclooxygenase COX-2 in the first step. As discussed in the **Introduction** to these pages, COX-2 is induced in endothelial and epithelial cells in response to a variety of stimuli. The effect of aspirin is to acetylate the enzyme, switching its catalytic activity (and its chirality) from prostanoid biosynthesis to production of 15*R*-HETE rather than the *S*-enantiomer. This is in turn converted to 5*S*,6*S*-epoxy-15*R*-hydroxy-EETE, as described above for lipoxins, by the action of the 5-lipoxygenase in leukocytes and thence to epi-lipoxins, i.e. epi-LXA<sub>4</sub> and epi-LXB<sub>4</sub> with 15*R*-stereochemistry. 15(*R*)-HETE produced by the action of a cytochrome p450 enzyme in the absence of aspirin can also be converted to 15-epi-lipoxins.

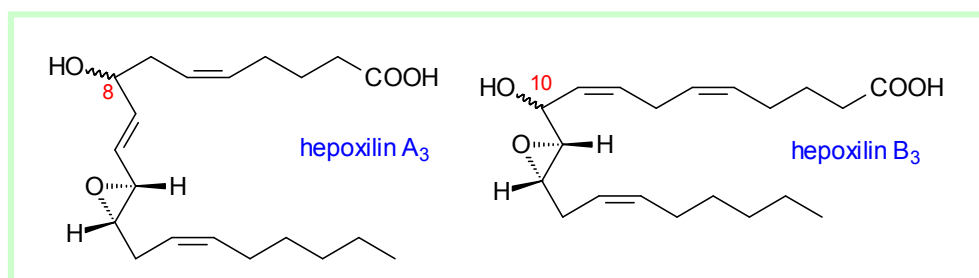


Aspirin thereby has the distinctive property of initiating the resolution of inflammation by stimulating the formation of mediators of the process much earlier than might have been expected. The distinctive lipoxins structures, which are conserved across species, are formed via cell-cell interactions, and they seem to act at both temporally and spatially distinct sites from other eicosanoids involved in the inflammatory responses.

Lipoxins are deactivated by the actions of 15-hydroxyprostaglandin dehydrogenase and prostaglandin reductase with production of 13,14-dihydro-15-hydroxy-LXA<sub>4</sub> and eventually 15-oxo metabolites. The epi-lipoxins have a two-fold longer half-life than the lipoxins as they are catabolized less efficiently.

#### 4. Hepoxilins

**Hepoxilins** are short-lived monohydroxy-epoxy eicosanoids produced in a number of organs or cell types and derived mainly from the product of 12-lipoxygenase action on arachidonic acid, i.e. 12*S*-hydroperoxy-5*c*,8*c*,10*t*,14*c*-eicosatetraenoic acid (12*S*-HPETE). They contain both hydroxyl and epoxy groups, the latter across the C11-C12 double bond, and unlike the leukotrienes and lipoxins, none of the double bonds are in conjugation.



12*S*-HPETE can either be reduced to the hydroxy compound (12*S*-HETE), or it can enter the hepoxilin pathway where it is acted upon by a hepoxilin synthase, which effects isomerization of

the hydroperoxide group The enzyme in skin is distinct from that in other tissues. Two hepxilins have been characterized, i.e. 8(*S/R*)-hydroxy-11*S*,12*S-trans*-epoxyeicosa-5*c*,9*t*14*c*-trienoic acid (hepxilin A<sub>3</sub> or HXA<sub>3</sub>) and 10(*S/R*)-hydroxy-11*S*,12*S-trans*-epoxyeicosa-5*c*,9*c*14*c*-trienoic acid (hepxilin B<sub>3</sub> or HXB<sub>3</sub>). Only HXA<sub>3</sub> is biologically active. The epoxide ring is labile and can be opened by an epoxide hydrolase to yield trihydroxy metabolites, termed 'trioxilins', which may also have some biological activity.

## 5. Biological Activities

As pro-inflammatory mediators, leukotrienes stimulate cellular responses that are quick in onset but do not last long, such as smooth muscle contraction, phagocyte chemotaxis, and increased vascular permeability, all of which are mediated via specific G-protein coupled receptors.

**Leukotriene B<sub>4</sub>** has an important function in the inflammatory process by its effect on leukocytes mediated via two G-protein-coupled receptors. It causes neutrophils to adhere to vascular endothelial cells and enhances the rate of migration of neutrophils into extra-vascular tissues, and it triggers several functional responses important for host defense, including the secretion of lysosomal enzymes, the activation of NADPH oxidase activity, nitric oxide formation, and phagocytosis. Also, it activates such intracellular signalling events as the mobilization of calcium, activation of phospholipases, the production of diacylglycerols and phosphoinositides, and the release of either anti- or pro-inflammatory agents, depending on circumstances. 5-Lipoxygenase and LTB<sub>4</sub> especially have been implicated in the chronic inflammation that is a part of the pathophysiology of atherosclerosis.

**Leukotriene C<sub>4</sub>**, together with LTD<sub>4</sub> and LTE<sub>4</sub> (the cysteinyl-leukotrienes, which jointly comprise the slow-acting substance of anaphylaxis), are known to exert a range of pro-inflammatory effects, including constriction of the airways and vascular smooth muscle, increasing plasma exudation and oedema, and enhanced mucus secretion. They are important mediators in asthma especially, but also in other inflammatory conditions, including cardiovascular disease, cancer, and gastrointestinal, skin, and immune disorders, again exerting their effects through two distinct G-protein coupled receptors. Some consider that it is the over-production of leukotrienes that is harmful rather than production *per se*. However, there is great interest currently in drugs that inhibit the effects of these lipids by functioning as agonists to their receptors.

There is a general impression is that leukotrienes produce harmful effects, especially in relation to the immune system and allergic diseases, such as asthma. However, there are suggestions that they may also be beneficial in that they stimulate the body's innate immunity against pathogens, including bacterial, fungal and viral infections, by promote the expression of mediators and receptors that are important for immune defense. For example, leukotriene B<sub>4</sub> can trigger the release of antimicrobial agents.

**Eoxins** have been implicated in inflammation of the airways in asthma patients, and in those with Hodgkin lymphoma, a malignant disorder with many characteristics of an inflammatory illness.

**Lipoxins** were the first eicosanoids to be discovered with a role in the resolution of inflammation, i.e. they are 'switched on' to limit the effects of inflammation. Indeed, they control the inflammatory response in such pathogenic conditions as asthma, arthritis, cardiovascular disorders, and gastrointestinal, periodontal, kidney and pulmonary diseases. Thus, they have opposing effect to LTC<sub>4</sub> and inhibit bronchial spasms. Like lipoxins, the aspirin-triggered **epi-lipoxins** also have potent anti-inflammatory actions, and this may provide further explanation for the efficacy of aspirin as a drug. It not only inhibits the synthesis of pro-inflammatory mediators but also induces the synthesis of anti-inflammatory ones. In particular, LXA<sub>4</sub> is produced endogenously and evokes protective effects via interactions with specific G protein-coupled receptors and a nuclear

transcription factor. All of the observed reactions appear to be highly stereo-selective in terms of double bond geometry and chirality of the hydroxyl groups. Lipoxins also have a regulatory role in the immune response to infection by parasitic pathogens, such as *Toxoplasma gondii*, and by *Mycobacterium tuberculosis*.

In the initial phase of inflammation, prostaglandin PGE<sub>2</sub> and other pro-inflammatory prostaglandins are produced. The signals that lead to the synthesis of such molecules in turn stimulate the transcription of enzymes required for the generation of lipoxins from arachidonate and the **resolvins** from fatty acids of the *omega*-3 family of fatty acids, which also have anti-inflammatory properties. The lipoxins are believed to function in promoting resolution by controlling the entry of neutrophils to sites of inflammation and the affected organs. They are also chemo-attractants for monocytes, i.e. cells that are required for wound healing. In effect, it appears that leukocytes are programmed to progress from pro- to anti-inflammatory responses, utilizing metabolites derived from both *omega*-6 and *omega*-3 fatty acids in the process. The possibilities for therapeutic intervention with such lipids to reduce the adverse effects of inflammation in various disease states are being actively explored.

**Hepoxilins** have pro-inflammatory properties in the skin, but anti-inflammatory in neutrophils. Most of the observed activities are associated with mobilization of calcium and potassium within cells or across membranes. In addition, hepoxilin A<sub>3</sub> is now known to be an important regulator of mucosal inflammation in response to infection by bacterial pathogens. Although lipoxygenase activity in brain tissues tends to be low, there is significant biosynthesis of hepoxilins in the pineal gland, which may be involved in the regulation of melatonin production.

### Recommended Reading

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