EICOSANOID Structures and Key Enzymes

1. Some Definitions

The term eicosanoid is used to embrace biologically active lipid mediators (C<sub>20</sub> fatty acids and their metabolites), including prostaglandins, thromboxanes, leukotrienes and other oxygenated derivatives, which are produced primarily by three classes of enzymes, cyclooxygenases (COX-1 and COX-2), lipoxygenases (LOX) and cytochrome P450 mono-oxygenases. The key precursor fatty acids are 8c,11c,14c-eicosatrienoic (dihomo-γ-linolenic or 20:3(n-6)), 5c,8c,11c,14c-eicosatetraenoic (arachidonic or 20:4(n-6)) and 5c,8c,11c,14c,17c-eicosapentaenoic (20:5(n-3) or EPA) acids (see our web page on ‘polyunsaturated fatty acids’). However, it is now impossible to discuss these compounds and their biological activities properly without also considering the docosanoids (resolvins and protectins) derived from 4c,7c,10c,13c,16c,19c-docosahexaenoic acid (22:6(n-3) or DHA), and related products formed by non-enzymatic means (isoprostanes). Similarly, plant products such as the jasmonates and other oxylipins derived from 9c,12c,15c-octadecatrienoic (α-linolenic or 18:3(n-3)) acid have analogous structures and functions. It is noteworthy that the precursor fatty acids for all of these belong to both the omega-6 and omega-3 families.

Of these fatty acids, arachidonic acid has been by far the most studied, and it is special in many ways. It is an essential fatty acid in that it cannot be synthesised de novo in animals, and linoleic acid from the diet is required as the primary precursor (as discussed elsewhere). As a major component of phospholipids, and especially of phosphatidylinositol, it is important for the integrity of cellular membranes. The four cis-double bonds mean that the molecule is highly flexible, and this helps to confer the correct degree of fluidity in the membranes. Diacylglycerols enriched in arachidonic acid and derived from phosphatidylinositol are important cellular messengers. Anandamide or N-arachidonoylthanolamine (see the appropriate webpage) is an endogenous cannabinoid or ‘endocannabinoid’, which produces neurobehavioral effects similar to those induced by cannabis and may have important signalling roles in the central nervous system, especially in the perception of pain and in the control of appetite. 2-Arachidonoyl-glycerol, also discussed elsewhere on this website, has similar properties. There are suggestions that arachidonic acid per se may have some biological importance in animal tissues; for example, the cellular level of unesterified arachidonic acid may be a mechanism by which apoptosis is regulated.

Arachidonic acid has only rarely been encountered in higher plants, but it is a constituent of some algae, fungi and moulds. In fungal infections of plants, it is known to elicit the production of plant defence compounds (phytoalexins), probably after conversion to oxygenated metabolites.

The oxygenated metabolites derived from arachidonic and related fatty acids are produced through a series of complex interrelated biosynthetic pathways sometimes termed the ‘arachidonate or eicosanoid cascade’. They are so numerous and have such a range of biological activities that they must provide a substantial component of the reason for the essentiality of the latter to the survival and well-being of animals. Documents in this series deal with each of the various classes of eicosanoids and related compounds. The structures of some examples of the important classes
are illustrated below. The **prostanoids** (prostaglandins, thromboxanes and prostacyclins) have distinctive ring structures in the centre of the molecule. The hydroxyeicosatetraenes are apparently simpler in structure, but are precursors for families of more complex molecules, such as the leukotrienes and lipoxins.

![Chemical structures](image)

The ‘natural’ eicosanoids are produced with great stereochemical precision, and this is essential for their biological functions. They are highly potent in the nanomolar range *in vitro* in the innumerable activities that have been defined, especially in relation to inflammatory responses, pain, and fever. They are produced in most organs and cell types, but with a high degree of tissue specificity, and even cooperatively between cells.

Biosynthesis of eicosanoids involves the action of multiple enzymes, several of which can be rate limiting. The figure below summarizes in simplistic terms the various pathways for the formation of eicosanoids. The first step in their biosynthesis is the production of free arachidonic acid in tissues from membrane phospholipids upon stimulation of the enzyme phospholipase A₂ by various physiological and pathological factors, including hormones and cytokines. There are then three main enzymatic pathways for eicosanoid formation, involving cyclooxygenases (COXs), lipoxygenases (LOs) and enzymes of the cytochrome P-450 family. The COX pathway (two isoforms denoted COX-1 and COX-2) produces the prostaglandins PGG₂ and PGH₂, which are subsequently converted into further prostaglandins, prostacyclin and thromboxanes (TXs).

There are several lipoxygenases that act upon different positions on the arachidonic acid, mainly 5, 12 and 15, although an 8-lipoxygenase is also relevant, to produce various hydroperoxy-eicosatetraenoic acids (HPETEs) and thence into hydroxyeicosatetraenoic acids (HETEs) and further products. For example, leukotriene LTA₄ is produced from 5-HETE and is in turn a precursor for LTB₄, cysteinyl-leukotrienes (CysLTs) and lipoxins (LXs). The cytochrome P-450 epoxygenase pathway produces hydroxyeicosatetraenoic acids and epoxides. It should be noted that many of the requisite enzymes, precursors and products are specific to particular types of cells.
There follows a brief of some of the key enzymes that are common to various biosynthetic pathways for eicosanoid production. Further discussion of these will be found in the web documents dealing with the various classes of eicosanoids.

2. Phospholipase A₂

Most of the arachidonic acid (and other polyunsaturated fatty acids) in animal tissues is in esterified form, mainly to phospholipids and phosphatidylinositol in particular. Before this arachidonate can be used for eicosanoid synthesis, it must be released by the action of the enzyme phospholipase A₂. In addition to phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine can be major substrates for arachidonic acid release, depending on the tissue and physiological conditions. The following is a brief summary. More details on the action of phospholipases can be found in the Animal Biochemistry section of this website.
A large number of enzymes with phospholipase A₂ activity have been characterized, and four main types have been identified that are relevant here – secretory, cytosolic Ca²⁺-dependent, cytosolic Ca²⁺-independent, and a peroxisomal Ca²⁺-independent. The first and third of these types show no specificity for arachidonic acid in particular, and they appear to have only a minor role in eicosanoid production (although secretory phospholipase A₂ may provide some arachidonate for cyclooxygenase-2 (see below)). Rather, they are involved in phospholipid re-modelling or general catabolism, where they ensure the availability of the required substrates. In addition, it is well known that further functions of these enzymes include digestion of dietary phospholipids and host defence against bacterial infections.

On the other hand, the cytosolic Ca²⁺-dependent phospholipase A₂ does have a marked specificity for phospholipids containing arachidonic acid in the sn-2 position, and there is clear evidence that the enzyme plays a key role in the release of this acid for generation of prostanoids and related metabolites. Indeed, it may be rate limiting for eicosanoid production in many tissues. It makes use of a catalytic Ser-Asp dyad to hydrolyse fatty acids, and it contains a so-called ‘C2’ domain that facilitates a calcium-dependent translocation from the cytosol to the membrane surface, where the phospholipid substrate is located. There are three isoenzymes in fact with molecular masses in the range of 60 to 100kDa. They are regulated by phosphorylation, and in the presence of low levels of calcium ions they can be translocated from the cytosol to the membranes of the nucleus and endoplasmic reticulum, where the precursor phospholipids and the key enzymes of eicosanoid biosynthesis are situated. In addition to control via transcriptional regulation, the activity of the cytosolic Ca²⁺-dependent phospholipase A₂ responds to various stimuli, such as hormones, cytokines and neurotransmitters. In particular, it has been demonstrated that ceramide-1-phosphate and phosphatidylinositol 4,5-bisphosphate bind to the enzyme. The latter is bound in a 1:1 stoichiometry and is required for activation and translocation of the enzyme to the site of action. The other product of the reaction, a lysophospholipid, may have signalling or regulatory functions.

The peroxisomal Ca²⁺-independent phospholipase has only recently been identified, but may be of particular importance for eicosanoid production in that it generates arachidonoyl species, such as 2-arachidonoyl lysophosphatidylcholine with high specificity.

The reverse reaction in which lysophosphatidylinositol is re-acylated also occurs. A membrane-bound O-acyltransferase (MBOAT7) specific for lysophosphatidylinositol with a marked preference for arachidonoyl-CoA has been characterized from neutrophils. This may be a means by which free arachidonic acid and eicosanoid levels are regulated.

3. Cyclooxygenases (Prostaglandin Endoperoxide H Synthases)

Cyclooxygenase-1 and cyclooxygenase-2 (COX-1 and COX-2), more correctly termed prostaglandin endoperoxide H synthases-1 and -2 (PGHS-1 and PGHS-2), are key enzymes that catalyse the first committed step in the synthesis of prostanoids from fatty acid precursors. COX-1 is always present in tissues, while COX-2 is induced by appropriate physiological stimuli (cytokines, tumor promoters and growth factors). The two iso-enzymes have about 60% homology in their amino acids, and are very similar in structure. They differ in that COX-2 has a larger pocket at the active site because of an isoleucine to valine substitution. The result is that in comparison with COX-1 it can be more permissive in utilizing fatty acids, such as dihomo-γ-linolenic and eicosapentaenoic acids in addition to arachidonic acid, as well as other lipid substrates (see below).

In humans, COX-1 and COX-2 are homodimers of 576 and 581 amino acids, respectively, and each has three mannose-containing oligosaccharides, one of which facilitates protein folding. A fourth oligosaccharide is found only in COX-2 and regulates its degradation. Each subunit of the dimer consists of three domains, the epidermal growth factor, the membrane binding domain, and
the substantial catalytic domain, which contains the two active sites on either side of a heme prosthetic group. They are integral membrane proteins of the endoplasmic reticulum and nucleus and are located on one side only of the bilayer.

Both enzymes catalyse the same two reactions. Thus, each carries out a cyclooxygenase reaction in which two molecules of oxygen are added to arachidonic acid to form a bicyclic endoperoxide with a further hydroperoxy group in position 15, i.e. to form prostaglandin PGG$_2$. The first reaction occurs at a hydrophobic channel in the centre of the enzyme, before the hydroperoxide intermediate is transferred to the heme-containing site on the surface of the enzyme where it is reduced by a peroxidase to form prostaglandin PGH$_2$. This is highly reactive and is the starting point for the biosynthesis of most other prostanoids (discussed in greater detail on a separate webpage).

Although the reactions occur at different sites, they are functionally coupled. The combined reactions are initiated by the oxidation of the heme group involved in the peroxidase reaction by traces of endogenous hydroperoxides with formation of a tyrosyl radical. This abstracts the 13-pro-S hydrogen from arachidonic acid and initiates the cyclooxygenase reaction, while during the reduction step the tyrosyl radical is regenerated so that activated COX can carry out multiple turnovers without a need to repeat the activation step. The other precursor polyunsaturated fatty acids interact with the enzymes in similar ways. As the catalytic tyrosyl radical can be transferred to an adjacent tyrosyl residue and become inactive after about 300 turnovers, the enzyme must be re-expressed constantly to generate metabolites.

The requirement for two distinct cyclooxygenases is not fully understood. In spite of the structural homology, separate genes encode COX-1 and COX-2 and they are regulated independently by different systems. The enzymes differ in their subcellular localization, substrate specificity and the manner in which they are coupled to upstream and downstream enzymes. In addition, the catalytic domains differ in structure, so that the susceptibilities to some inhibitors are not the same. It is now apparent that the two enzymes have different functional roles.

It has been suggested that COX-1 is used for ‘housekeeping’ (homeostatic) purposes, responding rapidly to circulating hormones, which require constant monitoring and regulation. It is a constitutive enzyme that produces prostaglandins in the endoplasmic reticulum, which exit cells and signal through G protein-linked receptors at the cell surface. However, there are also suggestions that it functions only at relatively high concentrations of arachidonic acid, for example during platelet aggregation, cell injury or acute inflammation. COX-1 is expressed at higher concentrations in those tissues where prostaglandins have specialized signalling functions, such as kidney, stomach, vascular endothelium, and especially blood platelets, where the enzyme provides precursors for thromboxane synthesis.
In contrast, COX-2 is an inducible enzyme that is not normally present in tissues other than the kidney and brain (where COX-2 is constitutive). It is expressed under the control of the pro-inflammatory transcription factor NF-κB in response to a wide range of extracellular and intracellular stimuli, such as cytokines, growth factors and tumor promoters, and produces prostanoids that are primarily pathophysiological or function during defined stages of cellular development. It is able to utilize much lower concentrations of arachidonic acid. COX-2 is especially important in cells that are involved in inflammation, such as macrophages and monocytes, and it is believed to be the form of the enzyme that has the main responsibility for the synthesis of those prostanoids involved in severe inflammatory states. Some of its products may modulate the transcription of certain genes in the cell nucleus. COX-2 is activated by hydroperoxide concentrations that are approximately tenfold lower than those that activate COX-1, raising the possibility that under limiting concentrations of peroxide, COX-2 may be fully active while COX-1 is not.

There is also a significant difference in the substrate requirements of the two iso-enzymes. While both utilize unesterified arachidonic acid as substrate, COX-2 can also metabolize dihomo-γ-linolenic and eicosapentaenoic acids. It can react with the endocannabinoid 2-arachidonoyl-glycerol to form 2-prostanoylglycerol derivatives, i.e. hydroxy endoperoxides analogous to PGH₂, which can be further metabolized by downstream synthases other than thromboxane synthase. Similarly, COX-2 is involved in conversion of anandamide (arachidonoyl ethanolamine) and arachidonoylglycerine to biologically active ‘prostamides’. These may simply serve as precursors of free prostanoids through hydrolysis, or there is increasing evidence that they may be a new class of lipid mediators with distinct biological properties of their own. The amide derivatives especially are relatively long-lived in plasma, and amides of PGF₂α are available as drugs to lower ocular pressure and treat glaucoma. 2-Prostanoylglycerol is subject to hydrolysis by esterases present in blood and some tissues. There is evidence of effects on calcium mobilization through distinct and novel receptors as well as activation of the PPARδ receptor.

Both COX iso-enzymes and thence prostaglandin synthesis are inhibited by non-steroidal anti-inflammatory drugs, such as aspirin (acetylsalicylic acid) and ibuprofen. Aspirin exerts this inhibition by binding to the cyclooxygenase site and transferring its acetyl group irreversibly to a specific serine residue. This protrudes into the active site and obstructs the binding of arachidonate. COX-1 is completely inhibited in this way, but COX-2 is only partially inhibited because of differences in the structures of the binding sites. All other drugs of this type exert their effects by reversible binding and competition for the active sites. The specific inhibition by aspirin is the reason for its well-known analgesic, anti-pyretic and anti-inflammatory effects as a pharmaceutical. Via its effect on COX-1, it inhibits thromboxane synthesis and thence platelet aggregation, and it is now recommended in cardiovascular therapy (the role of COX-2 in atherosclerosis is more complicated).

However, this does not fully explain aspirin's repertoire of anti-inflammatory effects, and it is now known to be intimately involved, through an action with COX-2, in the generation of oxygenated lipid mediators such as the ‘aspirin-triggered’ protectins (resolvins) and the epi-lipoxins, which exert profound anti-inflammatory effects. This may explain some of the clinical benefits of aspirin, especially in neuro-inflammation.

Synthesis of COX-2 is inhibited by steroidal anti-inflammatory drugs at the level of transcription. In addition, as the active site of COX-2 is smaller than that of COX-1, it has proved possible to develop a number of drugs that specifically inhibit the action of COX-2. As well as having analgesic and anti-inflammatory effects, these are used clinically to prevent cancer of the colon. However, some COX-2 selective inhibitors have been associated with an increased risk of cardiovascular disease and have been withdrawn from the market.
A prostaglandin H synthase isolated from the red alga *Gracilaria vertniculophylla* is very different in structure from its animal counterparts. However, it appears to function in a similar way, although it is not inhibited by non-steroidal anti-inflammatory drugs.

### 4. Lipoxygenases

Lipoxygenases are a family of enzymes that can be characterized as non-heme iron proteins or dioxygenases, which catalyse the abstraction of hydrogen atoms from a *bis*-allylic position of fatty acids while adding oxygen to generate hydroperoxide products. They occur widely in plants, fungi, some prokaryotes (cyanobacteria and proteobacteria) and animals, but not in the archaea and perhaps insects. The plant lipoxygenases have distinctive substrates and products, and they are described in our webpage dealing with plant oxylipins, although interesting parallels can be drawn with the mechanisms and functions of the animal enzymes. Similarly fungi and bacteria have distinctive lipoxygenases.

Animal lipoxygenases that utilize arachidonic acid as substrate are of great biological and medical relevance, because of the functions of the products in signalling or in inducing structural or metabolic changes in the cell. For example, they react with arachidonic acid *per se* to produce specific hydroperoxides and thence by downstream processing the plethora of eicosanoids, each with distinctive functions, which are described in these pages. However, they can also react directly with phospholipids in membranes to produce hydroperoxides that perturb the membrane structure. Thence, programmed structural changes in the cell can be induced, as in the maturation of red blood cells. In addition, phospholipid hydroperoxides can stimulate the formation of secondary products. Lipoxygenases can attack low-density lipoproteins directly with major implications for the onset of atherosclerosis.

![Diagram of lipoxygenase reactions](image)

The nomenclature of animal lipoxygenases is based on the specificity of the enzymes with respect to the products of the reaction with arachidonate; so for example, 12-LOX oxygenates arachidonic acid at carbon-12. The stereochemistry of the reaction can be specified when necessary (e.g. 12R-LOX or 12S-LOX), although the more important enzymic hydroperoxides have the S-configuration. Where more than one enzyme has the same specificity, it may be named after the tissue in which it is found, and there are platelet, leukocyte and epidermal types of 12-LOX, for example. Enzymes...
with specificities for four different positions occur in animal tissues, i.e. 5-LOX, 8-LOX, 12-LOX, and 15-LOX, although some of these have dual specificities, while many different isoforms exist in different species. There are considered to be six main lipoxygenase family members in humans and seven in mice. The positions at which the enzymes interact and the main products are illustrated in the figure.

Each of the lipoxygenase proteins in animal tissues has a single polypeptide chain with a molecular mass of 75–80 kDa. They have a N-terminal ‘β-barrel’ domain, which is believed to function in the acquisition of the substrate, and a larger catalytic domain containing a single atom of non-heme iron, which is bound to conserved histidine residues and to the carboxyl group of a conserved isoleucine at the C-terminus of the protein. For catalysis, the enzymes must be oxidized to the active ferric state.

All of the enzymes appear to include the fatty acid substrate within a tight channel with smaller channels that direct molecular oxygen toward the selected carbon, facilitating the formation of specific hydroperoxy-eicosatetraenes (HPETEs) and the corresponding hydroxy-eicosatetraenes (HETEs). In other words, the regiospecificity is regulated by the orientation and depth of substrate entry into the active site, while stereospecificity is controlled by switching the position of oxygenation on the reacting pentadiene of the substrate at a single active enzyme site, which is conserved as an alanine residue in S-lipoxygenases and a glycine residue in the rarer R-lipoxygenases. There is evidence that two amino acids opposite the catalytic iron determine the orientation of the substrate for entry into the enzyme channel. With 5-LOX and 8-LOX, the carboxyl group of arachidonic acid enters the active site, while with 12-LOX and 15-LOX the ω-terminal enters the site and facilitates the activity. It should be noted that the specificities of the enzymes are not always absolute and can differ between species.

Lipoxygenase action is believed to proceed in four steps - hydrogen abstraction (1), radical rearrangement (2), oxygen insertion (3), and peroxy radical reduction (4), all occurring under steric control, as illustrated.

For example, in the action of 5-LOX, the first and rate-limiting step is the abstraction of a hydrogen atom from carbon 7 by ferric hydroxide, involving a proton-coupled electron transfer in which the electron is transferred directly to the iron(III) to produce a substrate radical, while the iron atom is reduced to the ferrous form. The cis-double bond in position 5 migrates to position 6 with a change to the trans-configuration. The structure of this radical is uncertain, as are the details of the next steps in which the di-oxygen moiety (from molecular oxygen) is added, leaving the hydroperoxyl moiety in position 5. With the reduction step, the resulting product is 5S-hydroperoxy-
6,8c,11c,14c-eicosatetraenoic acid (5-HPETE). In the process, the iron atom is re-oxidized to its ferric form.

5-LOX is found only in cells derived from bone marrow (leukocytes, macrophages, etc) and is of particular interest as the product is the primary precursor for the leukotrienes. In contrast to other lipoxygenases, it requires the presence of a specific activator protein - lipoxygenase-activating protein (FLAP).

8-, 12- and 15-LOX operate in the same way to give analogous products. 15-LOX has a broader specificity, and in human leukocytes it is sometimes termed the 12/15-LOX (or 15-LOX-1) as it can also produce 12-HETE, 8,15-diHETE and eoxin A₄. It is able to oxidize linoleate to 13-hydroperoxy-octadecadienoate (and in part to the 9-isomer), and it differs from the others in that it can utilize arachidonate bound to phospholipids as a substrate, hence the interest in the role of the enzyme in membrane disruption and in disease states. Uniquely, it synthesizes both pro- and anti-inflammatory molecules. Mice do not express 15-LOX and only express the leukocyte-derived 12-LOX, so it can be difficult to extrapolate from animal experiments to human conditions.

Mouse skin produces an 8S-lipoxygenase that is structurally related to a second form of the human 15-lipoxygenase (15-LOX-2), but these have somewhat disparate tissue distributions and functions.

12-LOX from human platelets was one of the first lipoxygenases to be characterized, but a distinct isoform is present in the epidermis. Although the products generally have a hydroperoxide moiety in the S-configuration, some 12-lipoxygenases can produce the R-form. These are especially common in aquatic invertebrates, but they are also of considerable importance in mammalian skin. Indeed 12R-HETE was first characterized as a component of psoriatic lesions.

All the various lipoxygenases interact with arachidonic acid to form products that give rise to further eicosanoids with distinct biological properties. Also, these enzymes interact with the other essential polyunsaturated fatty acids of the omega-3 and omega-6 families to give comparable series of metabolites. These various lipoxygenase metabolites are discussed further elsewhere on this site, separately from the leukotrienes.

5. Catabolism of Eicosanoids

Efficient mechanisms for catabolism and deactivation of eicosanoids are essential for the regulation of their biological activities. While there are specific catabolic enzymes for thromboxanes and some leukotrienes, there is one major catabolic pathway that is common to most if not all other eicosanoids and is important for the control of their signalling activities. The first step consists in the oxidation of the 15(S)-hydroxyl group by a 15-hydroxyprostaglandin dehydrogenase, which exists in two forms with an NAD⁺-dependent enzyme displaying the greater activity. This enzyme metabolizes E-series prostaglandins, lipoxins, 15-HETE, 5,15-diHETE, and 8,15-diHETE, and probably many others to the corresponding 15-keto compounds. Incidentally, loss of this activity may influence the development of some cancers. The second step consists of reduction of the Δ13 double bond by a Δ13-15-ketoprostaglandin reductase (NADPH/NADH dependent). This was first identified as active against leukotriene B₄, but is now known to metabolize many prostaglandins and lipoxins.

Further catabolism of prostaglandins, HETES (except for 5-HETE) and lipoxins occurs by the beta-oxidation pathway common to fatty acids in general, i.e. via the carboxyl end of the molecule, leading to the formation of short-chain metabolites, which are excreted in the urine. Some of these eicosanoids are also excreted following glucuronidation. 5-HETE and leukotrienes undergo beta-oxidation from the omega-terminus following an initial omega-hydroxylation.
6. A General Comment

The chemistry, biochemistry, pharmacology, and molecular biology of eicosanoids are vast, complex and occasionally contradictory subjects that continue to develop extremely rapidly. These pages are intended only as a broad overview of the topic that can be understood by scientists with some knowledge of lipids in general but not of eicosanoids in particular. Those requiring a deeper insight should consult the papers cited below and in the further documents in this series.

7. Analysis

Eicosanoids tend to occur at low levels only in tissues. They have such a wide range of structures of varying stereochemistry that analysis has become a rather specialized task involving the use in the early years of gas chromatography linked to mass spectrometry but now increasingly of HPLC linked to electrospray mass spectrometry. Chiral chromatography also has an important role.

Recommended Reading

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