EICOSANOIDS AND RELATED COMPOUNDS – AN INTRODUCTION

Structures and Key Enzymes

1. Some Definitions

The term **eicosanoid** is used to embrace biologically active lipid mediators (C_{20} 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 epoxygenase. The key precursor fatty acids are 8*c*,11*c*,14*c*-eicosatrienoic (dihomo-γ-linolenic or 20:3(*n*-6)), 5*c*,8*c*,11*c*,14*c*-eicosatetraenoic (arachidonic or 20:4(*n*-6)) and 5*c*,8*c*,11*c*,14*c*,17*c*-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 related products produced by non-enzymic means (**isoprostanes**), and the **docosanoids** (resolvins) derived from 4*c*,7*c*,10*c*,13*c*,16*c*,19*c*-docosahexaenoic acid (22:6(*n*-3) or DHA). Nor can analogous plant products, such as the **jasmonates** and other oxylipins derived from 9*c*,12*c*,15*c*-octadecatrienoic (α-linolenic or 18:3(n-3)) acid, be ignored. It is noteworthy that the precursor fatty acids belong to both the *omega*-6 and *omega*-3 families.

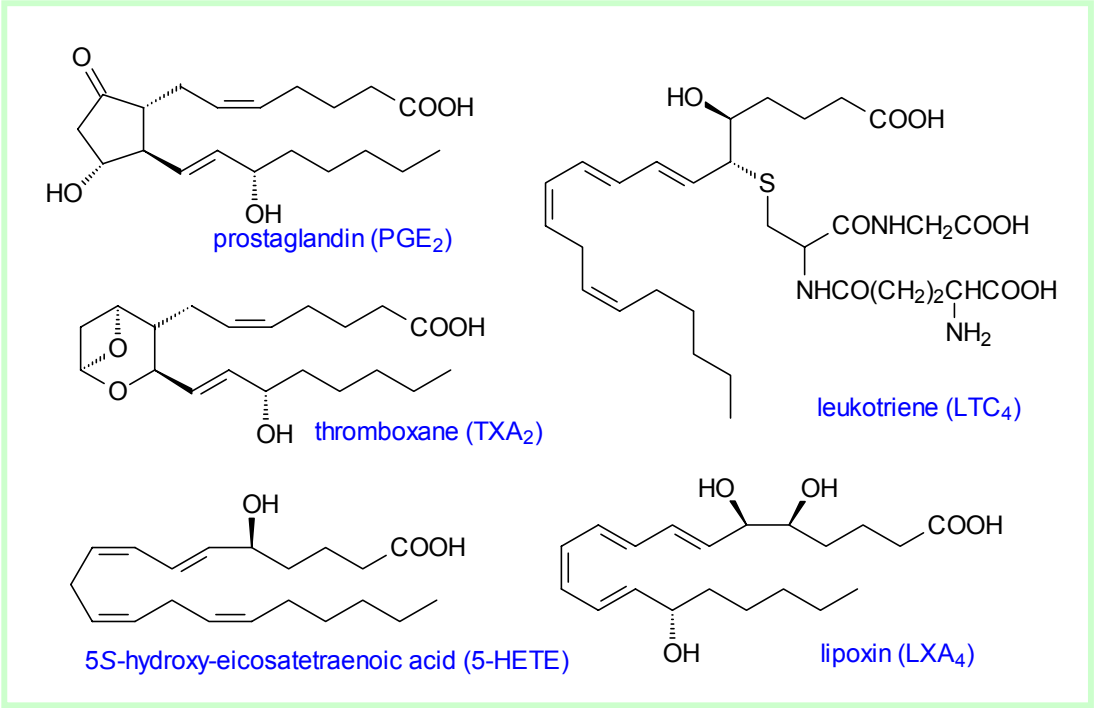
arachidonic acid

Of the precursor 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 a 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*-arachidonoylethanolamine (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 now 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 general 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 defense 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 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 will deal with each of the various classes of eicosanoids and related compounds. The structures of some examples of the important classes

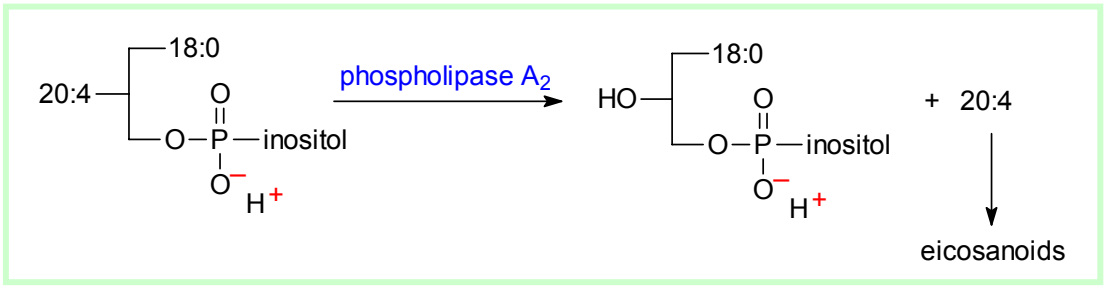
are illustrated below. The **prostanoids** (prostaglandins, thromboxanes and prostacyclins) have distinctive ring structures in the centre of the molecule. The hydroxyeicosatetraenes (HETE) are apparently simpler in structure, but are precursors for families of more complex molecules, such as the leukotrienes and lipoxins.



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. Biosynthesis of eicosanoids involves the action of multiple enzymes, several of which can be rate limiting. The following are key enzymes that are common to various biosynthetic pathways for eicosanoid production.

2. Phospholipase A_2

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 A_2.



A large number of enzymes with phospholipase A_2 activity have been characterized, and four main types have been identified – secretory, cytosolic Ca^{2+}-dependent and cytosolic Ca^{2+}-independent, and a peroxisomal Ca^{2+}-independent. The first and third of these types shows no specificity for arachidonic acid in particular, and they appear to have only a minor role in eicosanoid production (although secretory phospholipase A_2 may provide some arachidonate for cyclooxygenase-2 (see

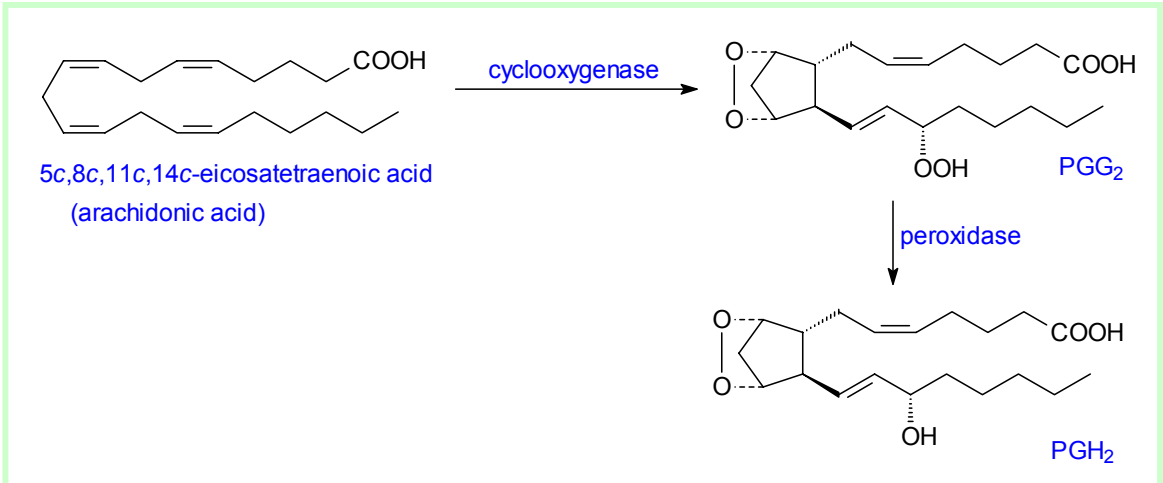
below)). Rather, they are involved in phospholipid re-modeling or general catabolism, where they ensure the availability of the required substrates.

On the other hand, the cytosolic Ca^{2+}-dependent phospholipase A_2 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. 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 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 Ca^{2+}-dependent phospholipase A_2 responds to various stimuli, such as hormones, cytokines, neurotransmitters, and so forth. In particular, it has been demonstrated that **ceramide-1-phosphate** binds to the enzyme and is required for activation and translocation to the site of action. The product of the reaction other than arachidonate, i.e. a lysophospholipid, may also have signalling or regulatory properties.

The peroxisomal Ca^{2+}-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.

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 are integral membrane proteins but are located on one side only of the bilayer. Both enzymes catalyse the same two reactions at different sites. 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. These reactions occur at a hydrophobic channel in the centre of the enzyme. The hydroperoxide is then reduced by a peroxidase reaction to form prostaglandin PGH_2, a reaction that occurs at a heme-containing site on the surface of the enzyme. PGH_2 is a highly reactive intermediate that is the starting point for the biosynthesis of most other prostanoids.

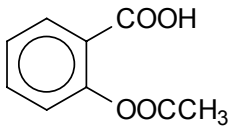


Although the reactions occur at different sites, they are functionally coupled. The combined reactions are initiated by the oxidation of the heme group involved in the peroxidase reaction by traces of endogenous hydroperoxides with formation of a tyrosine radical. This abstracts the 13-pro-*S* hydrogen from arachidonic acid and initiates the cyclooxygenase reaction. The other precursor polyunsaturated fatty acids interact with the enzymes in similar ways.

The requirement for two distinct cyclooxygenases is not fully understood. It has been suggested that COX-1 is used for 'housekeeping' purposes, responding rapidly to circulating hormones. 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-2 must be induced and produces prostanoids that function during defined stages of cellular development; it is able to utilize much lower concentrations of arachidonic acid. 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 use the endocannabinoid **2-arachidonoylglycerol** to form 2-prostanoylglycerol derivatives, i.e. hydroxy endoperoxides analogous to PGH_2, which can be further metabolized by downstream synthases. Similarly, COX-2 is involved in conversion of **anandamide** (arachidonoylethanolamine) and arachidonoylglycine 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 $PGF_{2\alpha}$ are available as drugs to lower ocular pressure and treat glaucoma.

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. Most 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. As it inhibits thromboxane synthesis and thence platelet aggregation, it is now recommended in cardiovascular therapy.



acetylsalicylic acid

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 lipid mediators such as the **epi-lipoxins**, which exert profound modulatory effects on the immune system, and **protectins**.

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.

4. Lipoxygenases

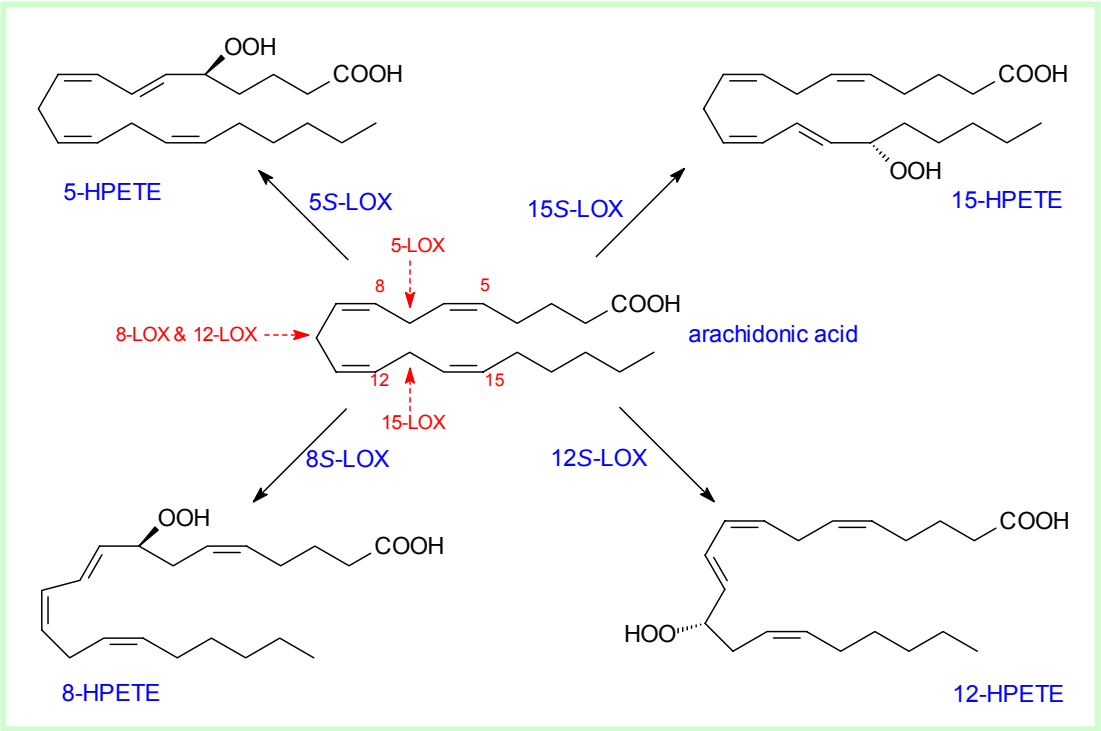
Lipoxygenases are a family of enzymes that can be characterized as non-heme iron proteins, which catalyse the abstraction of hydrogen atoms from a *bis*-allylic position of fatty acids while adding oxygen to generate hydroperoxide products. They occur widely in plants, fungi and animals,

but apparently not in plants and perhaps insects. The plant lipoxygenases have distinctive substrates and products and will be described elsewhere, although interesting parallels can be drawn with the mechanisms and functions of the animal enzymes. 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 will be 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 cells. In addition, the phospholipid hydroperoxides can stimulate the formation of secondary products. Lipoxygenases can attack low-density lipoproteins directly with major implications for the onset of atherosclerosis.

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 '□-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.

The nomenclature of lipoxygenases is based on the specificity of the enzymes with respect to their substrates, so for example 12-LOX oxygenates arachidonic acid at carbon-12. The stereochemistry of the reaction can be specified when necessary (e.g. 12*R*-LOX or 12*S*-LOX), although the 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.

Four main enzyme types with positional specificities occur in animal tissues, i.e. 5-LOX, 8-LOX, 12-LOX, and 15-LOX. The positions at which these enzymes interact and the natures of the main products are illustrated in the figure below. For example, In the action of 5-LOX, the first 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.



The structure of this radical is uncertain, as are the details of the next steps in which an oxygen atom is added, and the *cis*-double bond in position 5 migrates to position 6 with a change to the *trans*-configuration leaving the hydroperoxyl moiety in position 5. The resulting product is 5*S*-hydroperoxy-6*t*,8*c*,11*c*,14*c*-eicosatetraenoic acid (5-HPETE).

8-, 12- and 15-LOX operate in the same way to give analogous products. 15-LOX has a broader specificity and is able to oxidize linoleate to 13-hydroperoxyoctadecadienoate (and in part to the 9-isomer). It is also able to utilize arachidonate bound to phospholipids as a substrate, hence the interest in the role of the enzyme in membrane disruption and in disease states. 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 are also present in mammalian skin. There is particular interest in 5-LOX as the product is the primary precursor for the leukotrienes, but 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.

Within this family of enzymes, it is now believed that regiospecificity is regulated by the orientation and depth of substrate entry into the active site. Stereospecificity is apparently 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 *R*-lipoxygenases.

5. A General Comment

The chemistry, biochemistry, pharmacology, and molecular biology of eicosanoids are vast, complex and occasionally contradictory subjects that continue to develop at an extraordinarily rapid rate. 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.

6. 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 of primarily of gas chromatography linked to mass spectrometry. However, liquid chromatography in the chiral and other modes has an important role to play. The review by Tsikas cited below should prove a useful starting point for those wishing to learn more.

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Scottish Crop Research Institute (and Mylnefield Lipid Analysis), Invergowrie, Dundee (DD2 5DA), Scotland

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