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The Enzymes of the Human Eicosanoid Pathway

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Abstract

Eicosanoids are amphipathic, bioactive signalling molecules involved in a wide range of biological processes from homeostasis of blood pressure and blood flow to inflammation, pain, cell survival, and the progression of numerous disease states. The purpose of this review is to present an up-to-date and comprehensive overview of the enzymes of the eicosanoid pathway, their substrates, products, structure, isoforms, and regulation.

Keywords: Eicosanoid; Eoxin; Hepoxilin; Leukotriene; Lipoxin; Prostaglandin; Thromboxane

Abbreviations: 11R,12R-HxA_3: 11R,12R-hepoxilin A_3; 11R,12R-$TrXA_3$: 11R,12R-trioxilin A_3; 11R,12S-$TrXA_3$: 11R,12S-trioxilin A_3; 11R,12S-$TrXB_3$: 11R,12S-trioxilin B_3; 11S,12S-HxA_3: 11S,12S-hepoxilin A_3; 11S,12S-HxB_3: 11S,12S-hepoxilin B_3; HHT: 12-hydroxyheptadecatrienoic acid; 12-KETE: 12-oxoeicosatetraneoic acid; 12R-HETE: 12R-Hydroxyeicosatetraenoic acid; 12R-HPETE: 12R-hydroperoxyeicosatetraenoic acid; 12S-HETE: 12S-Hydroxyeicosatetraenoic acid; 12S-HPETE: 12S hydroperoxyeicosatetraenoic acid; 12S-HxB_3: 11S,12S-hepoxilin B_3; 15S-HETE: 15S-Hydroxyeicosatetraenoic acid; 15S-HPETE: 15S-hydroperoxyeicosatetraenoic acid; 5-epi-15HPETE: 5(6)-epoxy-15-hydroxyeicosatetraenoic acid; 5S-HETE: 5S-Hydroxyeicosatetraenoic acid; 5S-HPETE: 5S-hydroperoxyeicosatetraenoic acid; 9a,11 -α: 11 stereoisomer of PGF_2 ; AKR1B1: aldo-keto reductase 1B1; AKR1C3: aldo-keto reductase 1C3; ALOX12: Arachidonate 12(S)-lipoxygenase; ALOX12B: arachidonate 12(R)-lipoxygenase; LTC4S: leukotriene C4 synthase; ALOX15: arachidonate 15-lipoxygenase-1; ALOX15B: arachidonate 15-lipoxygenase-2; ALOX5: Arachidonate 5-lipoxygenase; ALOXE3: hydroperoxide isomerase; CBR1: carbonyl reductase 1;CLP: coactosin-like protein; CSF: cerebrospinal fluid; PKA: protein kinase A; DPEP: dipeptidase; DTT: dithiothreatol; EGF: epidermal growth factor; ERK2: extracellular signal-regulated kinase-2; EXA_4: eoxin A_4; EXA_4: eoxin A_4; EXC_4: eoxin C_4; EXD_4: eoxin D_4; EXE_4: eoxin E_4; FAM213B: prostamide/prostaglandin F synthase; FLAP: five-lipoxygenase activating protein; GDH: glutathione; GGT1: Gamma-glutamyl transaminase; GPX: glutathione peroxidases; GSNO: S-nitrosoglutathione; HPGDS: Hematopoietic prostaglandin D synthase; HPODE: 10(S)-hydroperoxyoctadecadienoic acid; INF: interferon gamma; SNP: single nucleotide polymorphism; iNOA: inducible NO synthase; LTA_4: leukotriene A_4; LTA4H: Leukotriene A-4 hydrolase; LTB_4: leukotriene B_4; LTC_4: leukotriene C_4; LTD_4: leukotriene D_4; LTE_4: leukotriene E_4; LXA_4: lipoxin A_4; LXB_4: lipoxins B_4; MAPEG: Membrane-Associated Proteins in Eicosanoid and Glutathione Metabolism; MAPKAPK2: Mitogen-Activated Protein Kinase-Activated Protein Kinase 2; MDA: Malonyl Dialdehyde; PDGF: Platelet-Derived Growth Factor; PGD2: Prostaglandin D_2; PGE_2: Prostaglandin E_2; $PGF_{2\alpha}$: prostaglandin $F_{2\alpha}$; PGG_2: Prostaglandin G_2; PGH_2: Prostaglandin H_2; PGI_2: Prostaglandin I_2; PLAT: Polycystin-1-lipoxygenase, alpha toxin; PAVSM: Porcine Aortic Vascular Smooth Muscle cells; PTGDS: Prostaglandin D_2 Synthase; PTGES: Prostaglandin E Synthase; PTGES2: Prostaglandin E Synthase-2; PTGES3: Prostaglandin E Synthase-3; PTGIS: Prostacyclin (PGI_2) Synthase; PTGS1: Prostaglandin G/H Synthase 1; PTGS2: Prostaglandin G/H Synthase 2; PUFA: Polyunsaturated Fatty Acid; TBXAS1: Thromboxane A Synthase 1; TGF: Transforming Growth Factor beta; TNF: Tumor Necrosis Factor alpha; TXA_2: Thromboxane A_2; TXB_2: Thromboxane B_2.

Introduction

Eicosanoids are amphipathic, bioactive signaling molecules derived from the oxidation of arachidonic acid and other similar polyunsaturated fatty acids (PUFAs). They are involved in a wide range of processes from homeostasis of blood pressure and blood flow, the resolution of inflammation, the perception of pain, cell survival, and the progression of numerous disease states. These biomolecules act most often as autocrine or paracrine signaling agents and most have relatively short half-lives. There are multiple subfamilies of eicosanoids, including the prostaglandins, thromboxanes, leukotrienes, lipoxins, resolvins, isoprostanes, and eoxins. Although the biological functions eicosanoids cover a wide range, one thing many have in common is context dependency. That is, stimulation by a particular eicosanoid in one tissue may in fact elicit a different and at times, opposite effect in another tissue.

Although there are a number of excellent reviews on various aspects of eicosanoid metabolism [1-5], this review was written to not only bring the subject up to date, but to do so in a highly comprehensive manner. In particular, each biosynthetic pathway for prostanoids, HETEs, lipoxins, hepoxilins, eoxins, and leukotrienes is discussed in detail. However, presentation of the entire eicosanoid pathway would be a vast undertaking. For this reason, the breadth of coverage has been purposely limited to derivatives of arachidonic acid. Thus, ω-3 resolvins, ω-6 linoleic acid, and ω-9 mead acid derivatives are not discussed. Further, epoxyeicosatrienoic acids, isoprostanes, furan-containing acids, and endocannabinoids have not been included to keep the volume of information within reasonable bounds.

Prostanoid Biosynthesis

Prostanoids are oxidized derivatives of arachidonic acid and consist of the following subclasses: 1) prostaglandins that are mediators of inflammatory and anaphylactic reactions, 2) thromboxanes that are mediators of vasoconstriction and platelet activation and 3) prostacyclins that are active in the resolution phase of inflammation. The prostanoid metabolic pathway is shown in Figure 1. Each prostanoid and associated enzymes are discussed in turn below and the properties for each are given in Table 1. The mRNA expression levels for each enzyme are described in detail in the supplemental data.

Enzyme	Common	UniProtKB	Gene	X-Ray	Reaction	#AA	MW kDa
Aldo-keto Reductase 1B1	AR	P15121	AKR1B1	1ADS	PGH2 → PGF2α	315	35.7
					PGH2 → PGD2		
Aldo-keto Reductase 1C3	PGFS	P42330	AKR1C3	1S1P	PGH2 → PGF2α	323	36.9
					PGE2 → PGF2α		
					PGD2 → 9a,11b-PGF2		
Arachidonate 12(R)-lipoxygenase	12R-LOX	O75342	ALOX12B	3D3L	AA → 5R-HPETE	701	80.4
Arachidonate 12(S)-lipoxygenase	12-LO	P18054	ALOX12	3D3L	AA → 12S-HPETE	663	75.7
					LTA4 → LXA4		
					LTA4 → LXB4		
Arachidonate 15-lipoxygenase-1	15-LOX	P16050	ALOX15	2ABT	AA → 15S-HPETE	661	74.7
					15S-HPETE → EXA4		
					LTA4 → 5-epi-15-HPETE		
Arachidonate 15-lipoxygenase-2	15-LOX-B	O15296	ALOX15B	4NRE	AA → 15S-HPETE	676	75.9
Arachidonate 5-lipoxygenase	5-LO	P09917	ALOX5	3O8Y	AA → 5S-HPETE	673	77.9
					5S-HPETE → LTA4		
					15S-HPETE → 5-epi-15-HPETE		
Carbonyl Reductase 1	CBR1	P16152	CBR1	1WMA	PGE2 → PGF2α	276	30.2
Dipeptidase	RDP	P16444	DPEP	1ITQ	LTD4 → LTE4	369	41.1
					EXD4 → EXE4		
Gamma-glutamyl transaminase	GGT 1	P19440	GGT	4GDX	LTC4 → LTD4	569	61.4
					EXC4 → EXD4		
Glutathione Independent Prostaglandin D Synthase	b-trace	P41222	PTGDS	3O19	PGH2 → PGD2	168	18.7
Hematopoietic prostaglandin D synthase	H-PTGDS	O60760	HPGDS	3EE2	PGH2 → PGD2	199	23.3
Hydroperoxide isomerase	e-LOX-3	Q9BYJ1	ALOXE3	none	12R-HPETE → 11S,12S-TrXA3	711	80.5
					12R-HPETE → 11S,12S-TrXB3		
					12S-HPETE → 12-KETE		
					12R-HPETE → 11R,12R-TrXA3		
Leukotriene A-4 hydrolase	LTA-4	P09960	LTA4H	1HS6	LTA4 → LTB4	610	69.2
Leukotriene C4 synthase	LTC4S	Q16873	LTC4S	2UUH	LTA4 → LTC4	150	16.6
					EXA4 → EXC4		

Prostacyclin Synthase	CYP8	Q16647	PTGIS	3B6H	PGH2 → PGI2	500	57.1
Prostaglandin E Synthase	mPGES-1	O14684	PTGES	4AL1	PGH2 → PGE2	152	17.1
Prostaglandin E Synthase-2	mPGES-2	Q9H7Z7	PTGES2	2PBJ	PGH2 → PGE2	377	41.9
Prostaglandin E Synthase-3	cPGES	Q15185	PTGES3	1EJF	PGH2 → PGE2	160	18.7
Prostaglandin G/H Synthase 1	COX-1	P23219	PTGS1	none	AA → PGH2	576	66
Prostaglandin G/H Synthase 2	COX-2	P35354	PTGS2	5F19	AA → PGH2	587	67.3
Prostamide/prostaglandin F synthase	FAM213B	Q8TBF2	FAM213B	none	PGH2 → PGF2α	198	21.2
Thromboxane A Synthase 1	TXS	P24557	TBXAS1	none	PGH2 → TXA2 PGH2 → 12-HHT + MDA	534	60.6
Note: # AA and MW reflect the processed polypeptide.							

Table 1: Selected Properties for Eicosanoid Enzymes.

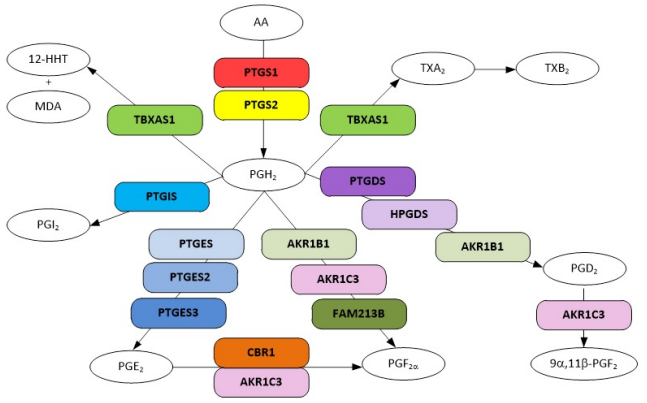

Figure 1: Prostanoid metabolism. Gene designations are given for the participating enzymes (rounded boxes) and accepted acronyms given for metabolites (ovals).

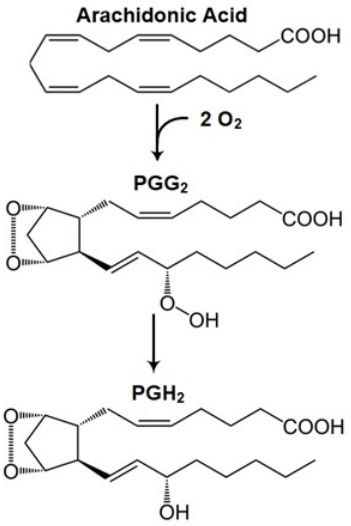

Figure 2: Reaction catalyzed by PTGS1 and PTGS2.

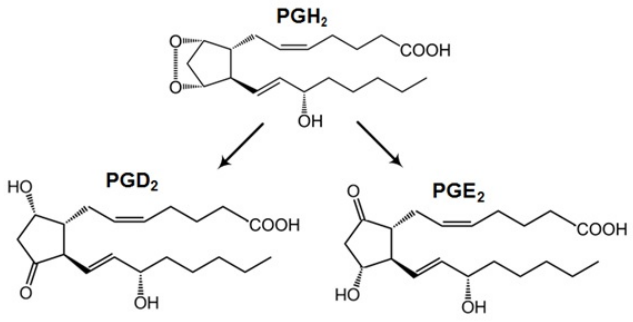

Figure 3: Synthesis of PGE_2 and PGD_2 from PGH_2.

Cyclooxygenases: Prostaglandin G/H synthase 1 and prostaglandin G/H synthase 2

Overview: Cyclooxygenase activity represents the first step in prostanoid biosynthesis whereby arachidonic acid is converted to the endoperoxide prostaglandin H_2 (PGH_2) via the transient prostaglandin G_2 (PGG_2) (Figure 2). PGH_2 is a multifunctional metabolite. First, and perhaps foremost, it serves as a precursor for the enzymatic synthesis of other prostanoids: PGI_2, PGE_2, $PGF_{2\alpha}$, PGD_2 and TXA_2. It also serves as a metabolic signal in its own right where it is involved in signaling for vasoconstriction [6,7] and platelet aggregation [8-10]. PGH_2 is a labile endoperoxide and it rapidly and non-enzymatically rearranges to both PGD_2 and PGE_2 (Figure 3). Further, these two metabolites in turn undergo ring cleavage to produce γ-keto aldehydes (levuglandin D_2 and E_2) which are highly reactive and form adducts with the ε-nitrogen on lysines that in turn can result in the crosslinking and aggregation of proteins. In particular, this function is known to accelerate the formation of $A\beta_{1\text{-}42}$ oligomers that are involves in Alzheimer's disease pathology [11].

There are two major protein isoforms of cyclooxygenase, Prostaglandin G/H Synthase 1 (PTGS1, COX-1, PGHS-1, PHS 1) and Prostaglandin G/H Synthase 2 (PTGS2, COX-2, PHS II, PGHS-2). In most tissues the former is expressed constitutively to maintain normal tissue function (e.g. maintain GI tract and renal function [12]) whereas the latter, normally present undetectable levels in most cells, is induced during inflammation. In contrast, in brain, testes and the *macula densa* of the kidney, both isoforms are expressed constitutively [13]. Structure and function of these isoforms are reviewed by Smith et al. [14,15] and more recently by Rouzer and Marnett [16] and Chandrasekharan and Simmons [17].

Human cyclooxygenase structure: PTGS1 and PTGS2 are heme containing, homodimers produced from distinctly different genes (human chromosome 9 and 1 respectively) [15,16]. They are membrane associated proteins (endoplasmic reticulum and nuclear) [18] with a unique membrane binding domain consisting of four short amphipathic helices [14,19]. The primary structure of PTGS2 shows 64.7% sequence homology to isoform-1 of PTGS1.

There are six known protein isoforms of Human PTGS1 (hPGHS1) [20]. For the sake of clarity all further references shall be made with respect to isoform-1 (UniprotKB-P23219-1). hPGHS1 is produced as a 599 residue polypeptide, cleaved to 576 residues (calculated as 65,996 Da) upon removal of the signal peptide. There are 10 known natural SNP variants (W8R, P17L, R53H, R149L, K185T, L237M, K341R, K359R, I443V, and V481I) [21,22]. Human PTGS2 (hPTGS2, UniprotKB-P35354) is produced as a 604 amino acid polypeptide, cleaved to 587 amino acids (calculated as 67,281 Da) upon removal of the signal peptide. There is only one known isoform and five reported natural SNP variants (R228H, P428A, E448G, V511A, and G587R) [22].

There are several X-ray structures reported for hPTGS2 (e.g. PDB entry 5F19) and no reported X-ray structured for hPTGS1. However, ovine PTGS1 shares a 92.5% sequence homology with hPTGS1 and thus the ovine structure (e.g. PDB entry 3N8V) may serve as a working model for the human protein structure.

Both hPGHS1 and hPTGS2 are N-glycosylated in a highly conserved manner. hPTGS1 is N-glycosylated at Asn-67, Asn-103 and Asn-143, producing glycoforms with yet-to-be determined composition [23]. Nemeth et al. [18] have used mass spectrometry to show that hPTGS2 is glycosylated at Asn-53, Asn-130, Asn-396, and Asn-580 producing three different glycoforms of molecular weight 71.4, 72.7, and 73.9 kDa. Interestingly, it has been shown that glycosylation at Asn-580 serves to signal degradation of hPTGS2 [24], in particular, transport to the cytoplasm for proteosomal degradation [25]. Further, Otto et al. [26] have shown that Asn-580 is glycosylated about 50% of the time in ovine PTGS2. There are two confirmed phosphorylations on hPTGS2 (Tyr-120 and Tyr-446) [27] and three predicted, but unconfirmed phosphorylations for hPTGS1 (Tyr-54, Thr-117, and Thr-220) (PhosphoSitePlus, https://www.phosphosite.org).

Regulation of cyclooxygenase: As noted previously, PTGS2 is an inducible form of cyclooxygenase in many tissues while PTGS1 is constitutive. Induction of PTGS2 transcription by growth factors and cytokines is well documented [28,29]. More recently, Cok and Morrison [30] have shown that PTGS2 expression is also regulated at the post-transcriptional level through the destabilizing 3' untranslatable region (nucleotides 1-60) of the mRNA which serves to significantly decrease the lifetime of the mRNA message.

Post translational regulation of PTGS2 has also been reported. Parfenova et al. [31] have shown that in pig cerebral microvesicles, PTGS2 and not PTGS1 is regulated by tyrosine phosphorylation, where the phosphorylation event serves to increase the activity of the enzyme. Alexanian et al. [27] have taken this a step further with PTGS2 in transfected human mesangial cells. Here they identified two kinases that phosphorylate two different tyrosines and used mass spectrometry to identify the sites of phosphorylation at Tyr-120 and Tyr-446. The former phosphorylation increases the average activity by 3-18% and the latter by 5-25%. The former site resides at the interface between the two subunits suggesting an allosteric effect on the activity and the latter is located in the catalytic domain and thus may have a more direct effect on the enzymatic activity.

S-nitrosylation of cysteine also serves to enhance the activity PTGS2. Kim et al. [32] have reported that inducible NO synthase (iNOS), a major mediator of inflammation, enhances the activity of PTGS2, a second prominent mediator of inflammation in a murine macrophage cell line. Activation requires binding of iNOS to a specific region of PTGS2 to efficiently facilitate nitrosylation of Cys-526, resulting in a two-fold enhancement of activity due solely to an increase in V_{max}.

Prostaglandin D synthase (PGDS)

Overview: Prostaglandin D_2 (PGD_2) is produced by mast cells, Th2 lymphocytes, and dendritic cells [33]. Binding to the CRTH2 receptor (prostaglandin DP_2 receptor) causes activation of Th2 lymphocytes, eosinophils and basophils, resulting in induced chemotaxis of Th2 lymphocytes and eosinophils as well as promoting cytokine production by Th2 lymphocytes. It is also a known sleep inducer in the central nervous system (CNS) [34,35].

Prostaglandin D Synthase (PGDS) represents two distinct types of small cytosolic glycoproteins involved in transport of lipophilic molecules such as bilirubin, retinal and retinoic acid and also catalyze the synthesis of PGD_2 from the substrate prostaglandin H_2 (PGH_2) (Figure 3) [36]. Both types are expressed in a variety of tissues with high amounts found in brain [37], heart [36], and testis [38,39]. The cellular distribution is quite ubiquitous and PGDS is found in the cytoplasm, endoplasmic reticulum, and nucleus [40]. The two distinct types of PGDS thus far reported are: 1) glutathione independent PGDS (PTGDS or L-PTGDS) [41,42], 2) glutathione requiring PGDS (spleen type, Hematopoietic prostaglandin D synthase (HPGDS or H-PTDGS)) [42,43].

Glutathione independent prostaglandin D synthase (PTGDS)

Overview: The brain type PTGDS exhibits a wide variety of CNS functions such as sedation and non-rapid eye movement sleep [35]. It is also involved in modulation of the immune response [44], inflammation response [45], and pain [46]. Also known as β-trace protein [37,47], the gene sequence indicates that it is a member of the lipocalin super-family [38] and further, it is the second-most abundant protein found in cerebrospinal fluid (CSF) [48].

Human PTGDS structure: Human PTGDS (hPTGDS) represents a collection of glyco-isoforms of a monomeric glycoprotein with molecular weights in the 27-34 kDa range [41,47]. 2D-polyacrylamine gel electrophoresis reveals seven distinct isoforms with isoelectric points from 5.8 to 7.5 [47]. Alterations in the isoelectric points of hPTGDS from CSF after treatment with neuramidase [38] and

identification of both mono and disialated oligosaccharides from recombinant hPTGDS suggest that at least some of the isoforms represent differences in the degree of sialation. In addition, differential phosphorylation may account for some of these isoforms as well [47,49,50]. Although specific functions for individual isoforms are presently unknown, Harrington et al. [47,51] and Pohl et al. [52] have shown that the relative amounts of each isoform normally associated with healthy individuals are altered in a variety of disease states.

There is one reported protein isoform of hPTGDS (UniprotKB-P41222) that is produced as a 190 amino acid transcript and processed to a 168 amino acid polypeptide (calculated as 18,698 Da) after removal of the signal peptide (residue 1-22) [48]. There is one known natural SNP variant (R56Q) and there are a number of X-ray structures available (e.g. PDB entry 3O19). The three-dimensional structure represents a classic lipocalin fold [53] where a single eight-stranded, continuously hydrogen-bonded antiparallel beta-barrel encloses the ligand-binding site [54]. Further, PTGDS is the only lipocalin that exhibits catalytic activity in addition to its lipid transporting capabilities. As noted above, hPTGDS is a highly glycosylated protein and contains both N- and O-glycosylations, some of which are sialated [38]. O-glycosylation at Ser-29 has been confirmed by Halim et al. [55]. There are numerous reports confirming N-glycosylation at Asn-51 [37,48,56,57] and at Asn-78 [38,48,56,58,59]. Grabenhorst et al. [60] have reported that 90% of the N-linked oligosaccharides are bientennary with terminal α-2,3 or α-2,6 sialations with a ratio of mono- to di-sialations of 1:5. Hoffmann et al. [56] reported similar findings with the exception that 40% were non-sialated, 40% monosialated and 20% disialated and have proposed a number of possible oligosaccharide structures. There are four predicted phosphorylations for hPTGDS (Tyr-107, Thr-147, Tyr-149, and Ser-150), none of which has been shown experimentally (PhosphoSitePlus, https://www.phosphosite.org).

Regulation of PTGDS: Regulation of PTGDS occurs on the transcriptional and post-translational modification levels as well as by proximity to other proteins. Tokudone et al. [61] have reported that expression of PTGDS in rat heart is enhanced by various glucocorticoids. Miyagi et al. [62] examined the effect of sheer stress at arterial levels on human vein endothelial cells and found that increased stress upregulates PTGDS mRNA. They also show that the effect is indirect through enhanced binding of activator protein-1 (AP-1) to the promoter region of the PTGDS gene.

Angenstein et al. [49] examined the activation of PTGDS by various kinases. They report that PTGDS responds only weakly to phosphorylation by phosphokinase A (PKA), but is a good substrate for casein kinase II (CK2). Specific sites for phosphorylation were not determined.

As described above, PTGDS has a number of known glycoforms and that the relative amounts of each is altered in various disease states [47]. It is unknown at this point if such changes in glycolysis modify the activity, alter the intracellular location or export rate, or alter the association with other proteins that may modulate PTGDS activity.

Hematopoietic prostaglandin D synthase (HPGDS)

Overview: Hematopoietic prostaglandin D synthase (HPGDS, H-PGDS) is expressed in a wide variety of tissues; however, the expression levels are species specific [63]. Urade et al. [64] have published an excellent review for this enzyme. Human HPGDS (hHPGDS) is expressed at high levels in the brain [63,65], heart [63] and immune system [63,66]. The gene sequence indicates that it is a member of the sigma glutathione S-transferase class of proteins. The relative abundance of hHPGDS in CSF is considerably lower than the other PGD_2 producing enzyme hPTGDS (1200 × lower) and is found at a concentration in CSF of 5-10 ng/mL [65].

Human HPGDS structure: Human HPGDS (hHPGDS) is a cytosolic [66] homodimer with a molecular weight in the 48 kDa range [64,67], although early reports noted it as a monomeric species [43]. The activity is enhanced by the presence of divalent cations where either Mg^{2+} or Ca^{2+} serve to activate and where the latter has a greater effect at lowering K_m and thus has a greater effect on the activity [68]. In addition, glutathione (GSH) is a required cofactor [64].

There is one reported protein isoform of hHPGDS (UniprotKB-O60760) that is produced as a 199 amino acid transcript that is not processed further, resulting in a polypeptide with a calculated molecular weight of 23,344 Da [63]. There are no known natural SNP variants. There is 1 potential acetylation site on hHPGDS, Lys-73, which has not been confirmed experimentally (PhosphoSitePlus, www.phosphosite.org). Numerous X-ray structures are available (e.g. PDB entry 3EE2).

Regulation of HPGDS: Although the regulation of many processes through the action of the HPGDS product PGD_2 have been reported, the regulation of HPGDS itself has not been studied in detail. However, Ghandi et al. [69] have reported that the bioavailability of selenium for incorporation into selenoproteins is required to upregulate the expression of HPGDS in human macrophages.

Prostaglandin E synthases

Overview: Prostaglandin E_2 (PGE_2) elicits a wide range of biological functions. It is most commonly associated with the mediation of inflammation and its role as a pro-inflammatory effector in the acute inflammatory response is well documented. PGE_2 acts as a vasodilator, facilitating the influx of mast cells, neutrophils and macrophages from the blood stream, leading to swelling and edema. At the same time, it stimulates neurons to increase the pain response and promotes pyrogenic effects [70,71]. PGE_2 also exhibits potent, context-dependent anti-inflammatory activities including inhibition of T-cell activation and IL-2 synthesis in and expression from T-cells [70,72-74].

PGE_2 is synthesized by a collection of Prostaglandin E synthases, all catalyzing the conversion of PGH_2 to PGE_2 (Figure 3), but each having a different structure and several having different cellular locations. Two of the known isoforms are glutathione requiring (PTGES, PTGES3) and one is glutathione independent (PTGES2).

Prostaglandin E synthase (PTGES)

Overview: Prostaglandin E Synthase (PTGES, microsomal prostaglandin E synthase, mPGES-1, MPGES1) is expressed in a variety of tissues and catalyzes the synthesis of PGE_2 from PGH_2 substrate. PTGES is constitutively expressed at low levels in lung, spleen, gastric mucosa, and kidney, but has not been detected in healthy heart, liver or brain [75]. PTGES is an inducible, glutathione-requiring enzyme [76,77] and is upregulated in response to inflammation triggers [78,79].

Human PTGES structure: Human PTGES (hPTGES) is a homotrimeric membrane-spanning protein and is a member of the membrane associated proteins in eicosanoid and glutathione metabolism (MAPEG) family of proteins [20,80,81]. There is one

reported protein isoform of hHPGES (UniprotKB-O14684) that is produced as a 152 amino acid transcript and not processed further, resulting in a polypeptide with a calculated molecular weight of 17,102 Da. No natural SNP variants have been reported. The crystal structure (e.g. PDB entry 4AL1 and 3DWW) reveals that each monomer is a four-helix bundle [20] and three subunits pack in a manner to provide a central cone-shaped cavity thought to be involved in substrate access. There is 1 potential phosphorylation site on hPTGES, Thr-34, which has not been confirmed experimentally (PhosphoSitePlus, www.phosphosite.org).

Prostaglandin E synthase-2 (PTGES2)

Overview: Prostaglandin E Synthase-2 (PTGES2, microsomal prostaglandin E synthase-2, mPGES-2) is expressed in a variety of tissues and catalyzes the synthesis of PGE_2 from PGH_2 substrate. PTGES2 is constitutively and strongly expressed in brain, heart, skeletal muscle, kidney and liver and expressed at lower levels in other tissues [82]. It is initially expressed as a membrane-bound Golgi protein, but is subsequently cleaved by beta amyloid precursor protein cleaving enzyme-1 (BACE-1) to a cytosolic truncated form that becomes enriched in the perinuclear region [83,84]. The protein has GSH and heme binding capacity, but neither is required for enzymatic activity. However, the presence of a thiol containing compound increases the activity [85]; dithiothreatol (DTT) is the most effective, but β-mercaptoethanol, GSH, or lipoic acid will also serve to activate the enzyme.

Human PTGES2 structure: Human PTGES2 (hPTGES2) is produced as a membrane-bound protein, possibly a dimer [85], and is a member of the GST superfamily. This polypeptide consists of 377 amino acids with a calculated molecular weight of 41,943 Da based on the sequence (UniprotKB-Q9H7Z7). There is only one known isoform and one reported natural SNP variant (R298H). The protein may also be subsequently cleaved to remove the first 87 residues of the N-terminus, producing a cytosolic product (33,107 Da) [83] which appears as a homodimer [85-87]. The crystal structure for hPTGES2 has yet to be reported, however, Heme and GSH-bound, truncated PTGES2 from *Macaca fascicularis* has been reported by Takusagawa et al. [87] (PDB entry 2PBJ). Since this protein shows a 97.6% sequence similarity to hPTGES2 it provides an excellent model for hPTGES2. Yamada et al. [85] have also presented the crystal structure for *Macaca fascicularis* PTGES2 (PDB entry 1Z9H) of the truncated form, but with bound indomethacin (IMN) to simulate bound substrate. Further, they have simulated the three-dimensional structure of the full transcript using secondary structure and hydropathy predictions for the first 87 residues and docking it with the truncated crystal structure. The resulting structure clearly shows the helix bundles that anchor each subunit to the membrane. There is one confirmed phosphorylation on hPTGES2 at Ser-95 [88] and 30 additional potential phosphorylation sites on hPTGES2, none of which has been confirmed experimentally (PhosphoSitePlus, www.phosphosite.org).

Prostaglandin E synthase-3 (PTGES3)

Overview: Prostaglandin E Synthase-3 (PTGES3, cytoplasmic PGES, cPGES, p23) is expressed in many tissues and is involved in a number of cellular processes. One such process is the production of PGE_2 from PGH_2 where PGH_2 resulting from the action of PTGS1 is preferred over PGH_2 derived from the action of PTGS2 due to cellular location and possible additional cofactors that may assist in the coupling of PTGS1 to PTGES3 [89]. A second function is the action of PTGES3 as a molecular chaperone in conjunction with Hsp90, modulating ribonucleoprotein telomerase [90] and disrupting receptor-mediated transcriptional activation by promoting disassembly of transcriptional regulatory complexes [91]. This protein is also involved with Hsp90 in the α-hydroxylation of proline on the hypoxia-inducible factor (HIF) which serves to mark HIF for degradation by the ubiquitin-proteosome pathway [92].

Human PTGES3 structure: Human PTGES3 (hPTGES3) is a monomeric, glutathione-dependent cytosolic protein belonging to the p23/wos2 family of proteins (UniProtKB-Q15185). Four protein isoforms have been reported, each formed via alternative splicing of the mRNA for isoform-1 [77]. Only isoform-1 (hPTGES3) has been isolated and characterized and thus will be the only hPTGES3 isoform discussed in detail here. There are no reported natural SNP variants. One X-ray structure is available for hPTGES3 (PDB entry 1EJF). hPTGES3 is a 160 amino acid polypeptide with a calculated protein molecular weight of 18,697 Da. Posttranslational modifications (discussed below) increase the molecular weight to the 23 kDa range.

Numerous posttranslational modifications (PTM) have been reported for hPTGES3. There are seven known phosphorylation sites Ser-44 [88,93], Ser-85 [88], Ser-113 [88,94-96], Ser-118 [95,97], Ser-148 [94-96], Ser-151 [93,94] and one inferred by similarity to the mouse counterpart (UniProtKB-Q9R0Q7, TEBP_MOUSE). SUMOylation of Lys-35 and Lys-65 has also been reported [98]. Lastly, Choudhary et al. [99] have identified the acetylation of Lys-33. The specific effects of these PTMs on catalysis, subcellular location, or protein association have yet to be determined.

Regulation of PTGES1, PTGES2, and PTGES3: PGE_2 serves a wide variety of physiological functions including, but not limited to vasodilation, gastric acid and mucus secretion, fever induction and inflammation. The response is dependent on cellular and tissue location and the associated receptors. For example, binding to the EP1 receptor stimulates bronchial constriction whereas binding to the EP2 receptor stimulates bronchial dilation. This wide variety of functions and varied cellular locations for each of the known PTGES enzymes leads one to suspect that each PTGES may have a specific function and may respond to different stimuli.

The differential response of PTGES1, PTGES2, and PTGES3 to pro-inflammatory stimuli is well documented. Stimulation of neurons by lipopolysaccharide (LPS) [79,100] and other pro-inflammatory stimuli leads to the induction of PTGES1, whereas PTGES2 and PTGES3 are produced constitutively [101]. Similarly, pulse acid treatment of Barrett's esophageal adenocarcinoma cell line FLO EA leads to induction of PTGES1, with no effect on PTGES2 and PTGES3 levels [102]. More specifically, pro-inflammatory cytokines have been shown to be involved in induction of PTGES1 [78]. Both IL-1β [103,104] and TNFα [105] induce PTGES1 and have no effect on PTGES2 and PTGES3 levels. In addition, PTGES1 is also induced by excess glutamate in ischaemic brain [106].

Substrate for each PTGES is produced by either or both PTGS1 or PTGS2. Several reports have shown that PTGES1 and PTGS2, known for their involvement in the inflammatory response are co-induced [104,105,106], confirming that the pair is directly involved in the production of PGE_2 in inflammation response. Mollerup et al. [107] have confirmed this relationship and have also shown that the expression PTGES3 and PTGS1 are coupled.

Differential expression of PTGESs has also been confirmed in cellular activities other than inflammation. Fujimori et al. [108] have

reported that the expression of mRNA of all three PTGES proteins is observed in adipocytes during adipogenesis. Using transfected siRNAs for each PTGES, only siRNA for PTGES1 reduced the production of PGE_2 (61.4%), indicating that PTGES1 is primarily responsible for production of PGE_2 in adipocytes, in particular, to suppress the early phase of adipogenesis. Nuttinck et al. [109] have shown that PTGES2 and PTGES3 are expressed constitutively in bovine oocytes whereas PTGES1 and PTGS2 are co-induced during an in vitro maturation. They further conclude that a transient induction of PGE_2 biosynthetic activity via the PTGS2/PTGES1 pathway during the maturation period increases the ability to produce progesterone from the immature to the fertilized stages. Sun et al. [110] have reported that PTGES1 mRNA levels increase upon treatment of mouse granulosa cells with human gonadotropin suggesting that PGE_2 synthesis by this enzyme may be important for follicular development, ovulation and luteal formation.

Prostacyclin synthase (PTGIS)

Overview: PGI_2 (prostacyclin) is well known for its regulatory role in the cardiovascular system where it is a potent vasodilator and inhibitor of platelet aggregation [111-113]. In addition, it is it is also established as a mediator of vascular permeability in response to acute inflammation and is known to elicit nociceptive pain response [111,113,114]. PGI_2 is quite unstable at physiological pH with a half-life of 2-5 minutes, forming biologically inactive 6-keto-prostaglandin F1α (6-keto-PGF1α) [111,115,116].

Prostacyclin Synthase (PTGIS, CYP8, CYP8A1) catalyzes the conversion of PGH_2 to prostaglandin I_2 (PGI_2) (Figure 4). PTGIS is constitutively expressed in endothelial cells and couples with PTGS1 [113,117] in the nuclear envelope [117]. However, it has also been shown to be upregulated from these levels with PTGS2 upregulation [112,118] in the endoplasmic reticulum [117,119]. PTGIS is also expressed in the CNS, mainly in neurons and to a lesser extent in glial cells. Here it is thought to be involved in the induction and maintenance of hyperalgesia by sensitization and may contribute to nociception in the CNS [2].

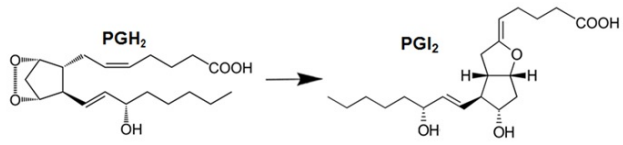

Figure 4: Reaction catalyzed by PTGIS.

Human PTGIS structure: PTGIS is a heme containing, monomeric, membrane-anchored enzyme belonging to the cytochrome P450 superfamily of proteins. Hydropathy analysis reveals a putative single helical amino terminal domain anchor that is typical for cytochrome P450 family members [120]. Human PTGIS (hPTGIS) is a 500 amino acid polypeptide with a calculated molecular weight of 57,104 Da (UniprotKB-Q16647). There are 8 known natural SNP variants (P38L, S118R, E154A, F171C, R236C, R375T, P500S) [121,122]. X-ray structures are available (e.g. PDB entry 3B6H).

It is well established that nitration of one or more tyrosines in PTGIS occurs *in vivo* (bovine and human) and serves to inhibit PTGIS [123-126]; Tyr-430 is the only confirmed nitration site [127]. There are also four potential phosphorylation sites on hPTGIS (Ser-52, Ser-200, Tyr-348, and Thr-399), none of which have been confirmed experimentally (PhosphoSitePlus, www.phosphosite.org).

Regulation of PTGIS: Expression of PTGIS is induced in concert with PTGS2 in response to pro-inflammatory cytokines, growth factors, bacterial endotoxins, tumor promotors, and various hormones secreted by immune cells [112,128-132]. It is noteworthy that the presence of even small amounts of PTGIS can prevent cytokine-induced cell death in insulin-producing islet cells [129].

Posttranlational regulation of PTGIS is facilitated via the reaction of Tyr-430 with peroxynitrite to form a 3-nitrotyrosine that inactivates the enzyme [128]; the source of peroxynitrite ($ONOO^-$) in vivo is the non-enzymatic reaction of nitric oxide (NO.) with the superoxide ion (O_2^{2-}) [127]. In cardiovascular systems, various inflammatory insults such as high glucose [123,125], tissue ischemia [124], and asthma or bronchitis [133] result in stimulation of the production of both nitric oxide and the superoxide ion, thus producing peroxynitrite which results in the inhibition of PTGIS. The lack of PTGIS activity results in the accumulation of the precursor PGH_2 that can then be converted to PGE_2 instead. Increased PGE_2 and a decrease in both PGI_2 and nitric oxide are known to promote the adhesion of white blood cells and their immigration to the inflammatory locus [126].

Prostaglandin F synthases

Overview: $PGF_{2\alpha}$ is one of the most abundant prostanoids in the brain and spinal cord [134-137] and is also found in many other tissues [113,138]. The functions are wide ranging and context dependent. $PGF_{2\alpha}$ is involved in inflammation as well as smooth muscle contraction, renal function, and blood pressure to name a few [113,133]. The stereoisomer 9α,11β-PGF_2 is thought to have similar functions [5,139].

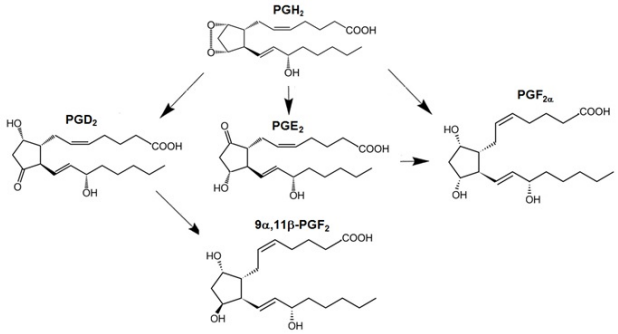

Figure 5: $PGF_{2\alpha}$ and9_α,11β-PGF_2 biosynthetic pathways.

Prostaglandin F Synthase represents a collection of enzymes that ultimately produce $PGF2_\alpha$ or the stereoisomer 9α,11β-PGF_2 (Figure 5). These enzymes fall into one of three different structural classes: 1) aldo-keto reductase superfamily (AKRB1, AKRC3), 2) the thioredoxin-like superfamily (FAM213B), and 3) the short-chain dehydrogenases/reductases (SDR) family (CBR1) [134,135,140-142]. Substrates for $PGF_{2\alpha}$ production are either PGH_2 or PGE_2 depending on the enzyme, whereas 9α,11β-PGF_2 is produced only from PGD_2. The properties of these and other NADH/NADPH-dependent reductases have been reviewed [143].

Prostamide/prostaglandin F synthase (FAM213B)

Overview: Prostamide/prostaglandin F synthase is a cytosolic protein that converts PGH_2 or PGH_2 ethanolamide to $PGF_{2\alpha}$ or $PGF_{2\alpha}$ ethanolamide respectively and requires NADPH as a co-enzyme [144,145]. Although the human enzyme has yet to be characterized, much is known about both the murine and porcine counterparts for which the human version shows a 88.4% homology to each [144]. Western and northern blot analysis, and enzymatic activity studies have shown that the porcine protein (A9CQL8) is found mainly in the brain and spinal cord [137,144]. Similarly, immunohistochemistry, double immunofluorescence, and immuno-electron microscopy confirmed the location of the murine version (Q9DB60) in the brain and that it is colocalized with myelin basic protein (MBP) in myelin sheaths but not in axons [137].

Human FAM213B structure: Human FAM213B (hFAM213B, UniprotKB-Q8TBF2) is a protein belonging to the Thioredoxin-like superfamily. Eight protein isoforms have been reported, each formed through alternative splicing of the mRNA for isoform-1 [77]; only isoform-1 has been identified at the protein level [89]. There are no reported natural SNP variants. hFAM213B consists of a single 198 amino acid polypeptide with a calculated molecular weight of 21,223 Da. X-ray structures have not been reported. The enzymatic activity of the porcine version increases four-fold in the presence of 1.5 M ammonium sulfate, suggesting that this enzyme may be dimeric or oligomeric [144].

There is one confirmed phosphorylation site on Tyr-108 [88]. The function of this modification is not yet known. There numerous additional potential phosphorylation sites that have not been confirmed experimentally (PhosphoSitePlus, www.phosphosite.org).

Regulation of FAM213B: Regulation of FAM213B remains to be fully elucidated. Its location in the myelin sheaths suggests that regulation is likely controlled by those factors involved in the formation and maintenance of myelin sheath [137,146].

Carbonyl reductase 1 (CBR1)

Overview: Carbonyl reductase 1 (CBR1) is a NADPH-dependent cytosolic protein with very broad substrate specificity and tissue expression [147-149]. The reaction germaine to this review is the reduction of PGE_2 to $PGF_{2\alpha}$ [141,147,150]. However, this enzyme is also involved in androgen metabolism, perhaps providing a functional link between the prostaglandin and the androgen pathways [141,151]. It is also involved in the reduction of quinones [150] and various xenobiotics, including several anti-cancer drugs [152,153], as well as reactive aldehydes such as 4-oxonon-2-enal which is believed to be involved in oxidative stress-related neurodegenerative disorders [147,154]. Interestingly S-nitrosoglutathione (GSNO) is also a substrate for human CRB1, implicating this enzyme in GSNO catabolism as well [147,155].

Human CBR1 structure: Human Carbonyl reductase 1 (hCBR1, UniProtKB-P16152) is a monomeric protein and a member of the short-chain dehydrogenases/reductases (SDR) family of proteins [147,155]. Three protein isoforms have been identified via Human cDNA [156], but only isoform-1 has been isolated as a protein transcript. In addition, two natural SNP variants have been reported, V88I and P131S, of which the former has been isolated and kinetic properties examined [157]. The biologically competent form of hCBR1, isoform-1, is a 277 amino acid polypeptide with a calculated molecular weight of 30,375 Da and has both NADPH and GSH binding sites [147]. There are several X-ray structures available (e.g. PDB entry 1WMA).

Several posttranslational modifications have been reported for hCBR1. Following cleavage of the initiating Met residue, Ser-2 is acetylated at its N-terminal [158]. Two phosphorylations have been predicted (UniProtKB) based on comparison to similar proteins, Ser-2 (CBR1_RAY) and Ser-30 (CBR1_MOUSE), but large scale phosphorylation studies have yet to confirm these predictions [158]. There are numerous potential phosphorylation, acetylation, ubiquitination sites on hCBR1 predicted by PhosphoSitePlus (www.phosphosite.org), none of which are confirmed experimentally.

Krook et al. [159] report an unusual Lys modification, N^6-(1-carboxyethyl) Lys of Lys-239, formed from the Lys and pyruvate through a Schiff base and subsequent reduction. This modification is specific for Lys-239, but is not quantitative, allowing for two different forms of the enzyme to exist. The modification is apparently not regulatory in nature nor a coenzyme adduct. The specific function, if any, remains to be elucidated. Similar findings of autocatalytic modifications have been presented by others [160,161].

As noted above, GSNO is a substrate for hCBR1. However, Hartmanova et al. [162] have shown that GSNO covalently modifies cysteines at positions 122,150, 226, and 227, and in a concentration dependent manner. These modifications alter the catalysis, increasing the k_{cat} for some substrates and decreasing the k_{cat} for others. Further, K_m is altered for most substrates to partially compensate for the change in k_{cat}, leading to overall small changes in catalytic efficiency.

Regulation of CBR1: There are numerous reports describing the transcriptional regulation of CBR1 in response to the presence of various xenobiotics (e.g. [163-165]), but few describing the effect on $PGF_{2\alpha}$ production. Ivanov et al. [166] show that CBR1 from Wistar-Kyoto rats is transcriptionally down-regulated by LPS or LPS activated cytokines, presumably through the inactivation of the Sp1 transcription factor suspected to be involved in the activation of CBR1 [166]. This would explain the observed downregulation of CBR1 in fever [157,166]. Guo et al. [140] report that cortisol enhances the transcription levels of CBR1 in human amnion fibroblast cells which may partly explain the concurrent increases of cortisol and $PGF_{2\alpha}$ in human amnion tissue with labor.

CBR1 is inhibited by a wide variety of compounds. For example, inhibition by the cardioprotectant Flavonoid 7-monohydroxyethyl rutoside (monoHER) [167], 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one (luteolin) [168], and (-) epigallocatechin gallate (EGCG) from green tea [169] have been demonstrated. Interestingly, hCBR1 is inhibited by a variety of both saturated and unsaturated fatty acids which may be involved in regulation of hCBR1 expressed in intestinal cells [170].

Aldo-keto reductase 1B1 (AKR1B1)

Overview: Aldo-keto reductase 1B1 (AKR1B1, AR) is a NADPH-dependent cytosolic protein with broad substrate specificity and tissue expression [171,172]. AKR family members metabolize aldehydes, monosaccharides, steroids, polycyclic hydrocarbons, as well as prostaglandins. AKR1B1 is the first enzyme in the polyol pathway where it converts glucose to sorbitol. The reaction relevant to this review is the conversion of PGH_2 to $PGF_{2\alpha}$ [171].

Human AKR1B1Structure: Human aldo-keto reductase 1B1 (hAKR1B1, UniProtKB-P15121) is a monomeric protein and a member of the aldo-keto reductase (AKR) family of proteins [171-173]. Only 1 protein isoform has been identified, however, six natural SNP variants have been reported (I15F, H42L, L73V, K90E, G204S, and T288I). hAKR1B1 is a 316 amino acid transcript, truncated to 315 residues (35,722 Da) upon removal of the initiator methionine [174,175]. This enzyme has both NADPH and substrate binding sites [172]. There are numerous X-ray structures available (e.g. PDB entry 1ADS).

Several posttranslational modifications have been reported for hAKR1B1. N^6-acetylations at Lys-95, Lys-222, and Lys-263 have been confirmed via LC-MS/MS analysis [99]. The degree and function of acetylation at these positions has yet to be determined. One phosphorylation at Ser-3 has been predicted (UniProtKB) based on comparison to the rat protein (CBR1_RAT). Large scale phosphorylation studies have yet to confirm this prediction [158]. Following cleavage of the initiating Met residue, Ala-2 has been predicted to be acetylated at its N-terminal, based on the observations for porcine AKR1B1 [174], but this too has not been confirmed experimentally [99]. Numerous phosphorylations, additional acetylation, ubiquitination, methylation and one S-nitrosylation site (Cys-299) are predicted by PhosphoSitePlus (www.phosphosite.org), none of which have been shown experimentally.

Regulation of AKR1B1: Induction of hAKR1B1 mRNA in adipose cells by the inflammatory cytokines TNF-α and IL-1β has been demonstrated [176]. Interestingly, expression levels for hAKR1C3, an alternative enzyme producing $PGF_{2\alpha}$ from PGH_2, is unaffected by the inflammatory cytokines, indicating that cytokine-stimulated $PGF_{2\alpha}$ synthesis in adipocytes is predominantly due to the action of hAKR1B1. In stereogenic cells of mouse adrenal tissue, AKR1B1 is induced in the presence of Adrenocorticotropic hormone (ACTH), suggesting that the gene is under cAMP/ACTH control. In porcine endometrial tissue the expression of AKR1B1 is increased by both estrogen and IL-1β.

Aldo-keto reductase 1C3 (AKR1C3)

Overview: Aldo-keto reductase 1C3 is a NADPH-dependent cytosolic protein with with broad substrate specificity and tissue expression [135,175,177-181]. This enzyme metabolizes aldehydes, steroids, and prostaglandins [175,179-181]. Of particular interest here is the multi-functional redox behavior towards select prostaglandins. This enzyme catalyzes the reduction of PGD_2 to 9α,11β-PGF2 as well as the reverse reaction and is also capable of converting PGH_2 to $PGF_{2\alpha}$ and PGE_2 to $PGF_{2\alpha}$ [182].

Human AKR1C3 structure: Human aldo-keto reductase 1C3 (hAKR1C3, UniProtKB-P42330) is a monomeric protein and a member of the Aldo-keto reductase family (AKR) family of proteins [182,183]. Two protein isoform have been identified, however, only isoform-1 (hAKR1C3) has been obseved at the protein level [77,182]. In addition, six natural SNP variants have been reported (H5Q, R66Q, E77G, R170C, M175I, and P180S) [184]. hAKR1C3 is a 323 amino acid polypeptide with a calculated molecular weight of 36,853 Da with both NADPH and substrate binding sites [172,185]. Many X-ray structures are available (e.g. PDB entry 1S1P). There are 31 potential phosphorylation sites on hAKR1C3, none of which have been confirmed experimentally (PhosphoSitePlus, www.phosphosite.org).

Regulation of AKR1C3: Induction of AKR mRNA in adipose cells by the inflammatory cytokines TNF-α and IL-1β has been examined by Michaud et al. [176]. Interestingly, the expression level for hAKR1C3 is found to be low and unaffected by the inflammatory cytokines, whereas the expression of hAKR1B1 is markedly increased. They concluded that the cytokine-stimulated $PGF_{2\alpha}$ synthesis in adipocytes is predominantly due to the action of hAKR1B1 and suggest that the function of hAKR1C3 in these cells is the reduction of ketosteroids. Dozier et al. [186] have reported that treating monkey granulosa cells with human chorionic gonadotropin (hCG) increases the expression of AKR1C3 as well as AKR1C1 and AKR1C2 that peaks in 24-36 hours. The results suggest that all three control follicular $PGF_{2\alpha}$ levels during the periovulatory interval.

Thromboxane A synthase 1 (TBXAS1)

Overview: Thromboxane A_2 (TXA_2) is a potent vasoconstrictor [187] and platelet activator [188,189], quite the opposite of PGI_2. In fact, the TXA_2/PGI_2 balance is thought to be central to maintaining healthy cardiovascular status [190,191]. TXA_2 is not only involved in platelet activation, but also in recruitment of more platelets to the primary plug [187,188].

Thromboxane A_2 (TXA_2) is produced from PGH_2 through the action of thromboxane A_2 synthase 1 (TBXAS1, TXA synthase, TXS). It is an unstable metabolite with a half-life on the order of 30 seconds [113] and hydrolyzes to the biologically inactive thromboxane B_2 (TXB_2) (Figure 6). In addition, TBXAS1 simultaneously forms 12-hydroxyheptadecatrienoic acid (HHT) and malondialdehyde (MDA) in equal molar amounts compared to TXA_2; HHT is a known leukotriene B4 receptor 2 (BLT2) agonist. It is noteworthy that the primary metabolite of HHT, 12-oxoheptadeca-5(Z)-8(E)-10(E)-trienoic acid (Oxo-HT), is a known inhibitor of platelet aggregation acting as a TXA_2 receptor antagonist [192]. TBXAS1 is primarily expressed in platelets, but is found in other cells and tissues such as macrophages, lung fibroblasts, lung, spleen, brain and polymorphonuclear leucocytes [191,193-195]. The primary source for platelet PGH_2 substrate has been shown to be the constitutively expressed PTGS1, however, in marcophages the inducible PTGS2 is the primary source [113,190,196].

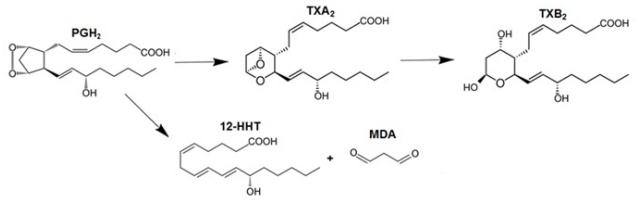

Figure 6: Reactions catalyzed by TBXAS1.

Human TBXAS1 Structure: Human TBXAS1 (hTBXAS1, UniProtKB-P24557) is a monomeric, heme-requiring transmembrane protein and a member of the cytochrome P450 superfamily. hTBXAS1 is a 534 amino acid polypeptide with a calculated molecular weight of 60,649 Da. There are five known isoforms, but only one isoform has been observed at the protein level. There are 30 reported natural SNP variants [122,197-206]. Although the sequence for the primary isoform given by UniProtKB is the P24557 isoform, all cited references and other publications refer to the sequence given for HCG14925, isoform

CRA_a (Q53F23) [197,201,207-209]. The only difference between the two is the initiator Met on Q53F23. No X-ray structures for hTBXAS1 are currently available.

There are no posttranslational modifications for hTBXAS1 reported to date. However, there are five potential N-glycosylation sites at Asn-56, Asn-104, Asn-107, Asn-185, and Asn-303. There are also two potential phosphorylation sites, Thr-58 and Ser-142 (PhosphoSitePlus, www.phosphosite.org).

Regulation of TBXAS1: In most normal, healthy tissue TBXAS1 is constitutively expressed and the production levels for TXA_2 and TBX_2 are dependent only on the availability of PGH_2 substrate [118,119,210,211]. The situation in the uterus is somewhat different. In myometrial smooth muscle, TBXAS1 expression is higher at term pregnancy than preterm and in specimens from labor as compared to that from non-labor specimens [212]. The results suggest that TXA_2 may play a role in initiation and progression of labor in women. In contrast, TBXAS1 expression is low in stromal cells and independent of the reproductive phase. As a second example, TBXAS1 expression in atherosclerotic lesion areas increases during the progression of atherogenesis [213,214].

There are also conflicting reports regarding the effect of peroxynitrite formed from nitric oxide on the activity of TBXAS1. Several reports indicate that peroxynitrite enhances the production of TXA_2 and thus TXB_2 through the inhibition of PTGIS, a competitor for the same PGH2 substrate [125,198]. On the other hand, there are several reports that nitric oxide or nitric oxide donors such as 6-(2-hydroxy-1-methyl-2-nitrosohydrazino)-N-methyl-nitroso-hydrazine (MMNN) directly inhibit TBXAS1 [215-217].

HETEs, Lipoxins, Hepoxilins, Trioxilins, Eoxins, and Leukotriene Biosynthesis

Overview

The biosynthesis of hydroxyeicosatetraenoic acid (HETE), Lipoxins, and hepoxilins are accomplished by a group of structurally similar enzymes of the lipoxygenase family.

The overall metabolic pathway is shown in Figure 7 and the properties for the enzymes involved are given in Table 1. The mRNA expression levels for each enzyme are described in detail in the supplemental data. The HETE family of molecules and their unstable precursors, the hydroperoxyeicosatetraenoic acids (HPETEs), are notable as precursors for the Leukotriene family, Lipoxin family and Eoxin family of metabolites, as well as serving as signaling molecules in their own right [218]. For example, 12(S)-HETE and 15(S)-HPETE are involved in monocyte binding in the vasculature [219,220]. Both 5-HPETE and 12(S)-HPETE are involved in modulating neurotransmission whereas their hydroacid products have no effect [221]. Further, both 12(S)-HETE and 15(S)-HPETE are involved in cell survival mechanisms [222].

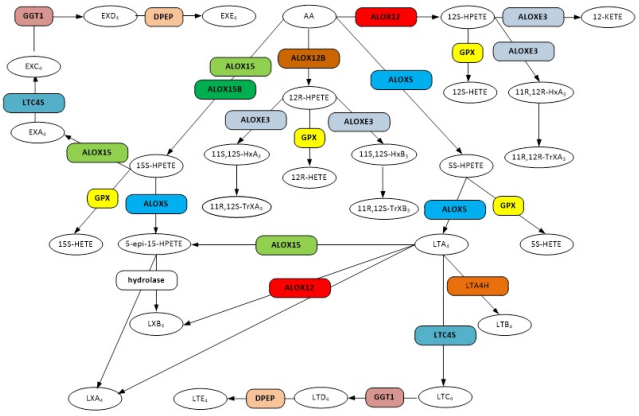

Figure 7: Metabolic pathways for Hydroxyeicosatetraenoic acids (HETEs), Lipoxins, Hepoxilins and Leukotrienes. Gene designations are given for the participating enzymes (rounded boxes) and accepted acronyms given for metabolites (ovals).

Lipoxins represent a family of metabolites that are either directly or indirectly produced by lipoxygenases. Lipoxin A_4 (LXA_4) and lipoxin B_4 (LXB_4) induce anti-inflammatory and pro-resolution mechanisms including repression of leukocyte-mediated injury and pro-inflammatory cytokine production, as well as inhibition of cell proliferation and migration [223,224]. These lipoxins are potent activators of monocytes through stimulation of chemotaxis and adherence [225, 226].

Hepoxilin A_3 (HxA_3) and B_3 (HxB_3) are unstable epoxy alcohol derivatives of arachidonic acid that are readily converted non-enzymatically to their acidic triol counterpart known as Trioxilin A_3 ($TrXA_3$) and Trioxilin B_3 ($TrXB_3$) respectively [227]. Hepoxilins are known to cause mobilization of intracellular calcium in human neutrophils and cause plasma leakage [228]. They also serve as chemo-attractants. For example, synthetic and naturally occurring gradients of HxA_3 have been shown to drive significant numbers of neutrophils across epithelial barriers, whereas eosinophils fail to respond to these gradients [229]. $TrXA_3$ and $TrXC_3$ and HxA_3 are endogenous vascular relaxing factors that act as thromboxane antagonists and thus help regulate vascular homeostasis [230].

Lipoxygenases

Overview: Lipoxygenases represent a non-heme, iron containing class of enzymes that oxygenate an array of PUFA substrates [reviews: 231,232]. Although the focus here is on the oxygenation of arachidonic acid and derivatives thereof, other PUFAs such as linoleic acid are also substrates for some lipoxygenases [233-235]. Lipoxygenases are found in plants, animals, fungi and cyanobacteria, but rarely found in other prokaryotes [236].

Each reaction involves a stereospecific (pro-R or pro-S) hydrogen abstraction followed by a stereospecific addition (R or S) of oxygen and subsequent reduction to the hydroperoxyeicosatetraenoic acid (HPETE). Hydrogen abstractions always occur on the methylene between two double bonds and subsequent addition of oxygen two carbons away. Throughout nature there are lypoxygenases that are specific for 10 of the 12 possible positions on the arachidonic acid substrate: 5R, 5S, 8R, 8S, 9R, 11R, 11S, 12R, 12S, and 15S [237]. Five of

these have been identified in humans: 5S, 12R, 12S, and two different 15S [232,234,238,239]. All known reactions proceed through a series of steps: 1) hydrogen abstraction, 2) radical rearrangement, 3) oxygen insertion, and 4) peroxy radical reduction (Figure 8) [236]. It has also been shown that the enzymes require a small amount of oxidized lipid to achieve maximal enzymatic activity through oxidation of the iron from the ferrous to ferric state [239-243]. The range of activities is achieved through specific amino acids in the active site that promote substrate entrance via the carboxyl end or methyl end and precise positioning of the fatty acid carbon chain in the active site [234,235,244].

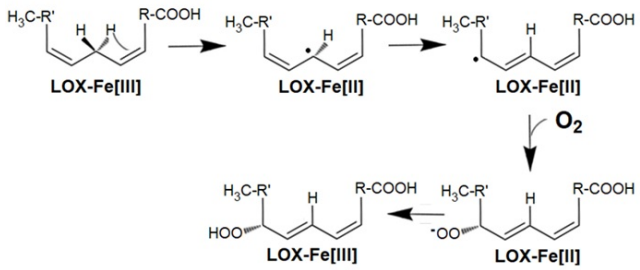

Figure 8: Elementary reactions involved in the lipoxygenase reaction. Rendered from Ivanov et al. [236].

The lipoxygenase structure consists of an N-terminal β-barrel structure known as PLAT (Polycystin-1-lipoxygenase, alpha toxin) domain, and a larger α-helical catalytic domain [234]. It is the former that, upon calcium binding, exposes hydrophobic residues and the region becomes a lipid anchor, allowing the cytosolic protein be become membrane associated [234,245-248].

Arachidonate 15-lipoxygenase-1 (ALOX15)

Overview: Mammalian ALOX15 (15-LOX, 15-LOX-1) is a monomeric cytosolic protein that becomes membrane associated in the presence of calcium [234,249,250]. ALOX15 is the major lipoxygenase found in reticulocytes [233,235], but is also expressed in leukocytes [251,252], heart tissue [253], and airway epithelial cells [254].

Both substrate and product specificity are diverse (Figure 9). ALOX15 converts arachidonic acid into 12(S)-hydroperoxyeicosatetraenoic acid (12S-HPETE) and 15(S)-hydroperoxyeicosatetraenoic acid (15S-HPETE) in a ratio of 1:9 [233,235] and also converts linoleic acid to 13(S)-hydroperoxyoctadecadienoic acid (13S-HPODE) and 10(S)-hydroperoxyoctadecadienoic acid (10S-HPODE) [234,236,251]. Eicosatrienoic acids may also serve as substrates [233]. In addition, ALOX15 not only oxygenates free fatty acids, but also membrane phospholipids when in the membrane bound state [249] and also generates 15-HETE conjugated to phosphatidylethanolamine, an intracellular signaling molecule [254].

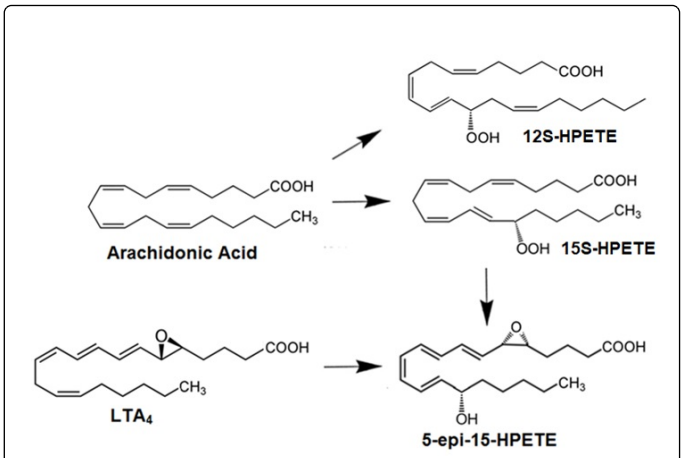

Figure 9: Reactions of 15-lypoxygenase.

An additional substrate for ALOX15 is the ALOX5 (see below) product leukotiene A_4 (LTA_4) where the epoxide product 5(6)-epoxy-15-hydroxyeicosatetraenoic acid (5-epi-15-HPETE) is formed as a precursor to the synthesis of lipoxin A_4 (LXA_4) and lipoxin B_4 (LXB_4) [255-258].

Human ALOX15 structure: Human ALOX15 (hALOX15, UniProtKB-P16050) is a monomeric, non-heme iron protein and a member of the lipoxygenase family. There are two known isoforms [77], but only one isoform has been observed at the protein level. In addition, there are 7 natural SNP variants (D90H, G102V, N103K, R205Q, V239M, A461P, and T560M) [22,259]. hALOX15 is translated as a 662 amino acid transcript and posttranslationally modified by removal of the initiating methionine, producing a 661 amino acid polypeptide with a calculated molecular weight of 74,673 Da [260]. One X-ray structure is available (PDB entry 2ABT).

No specific posttranslational modifications for hALOX15 have been reported with the exception of the removal of the initiator methionine [260]. However, two isoforms for hALOX15 have been isolated [251]. The only apparent difference between the two is chromatographic behavior, suggesting that a posttranslational modification is responsible for the difference. PhosphoSitePlus (https://www.phosphosite.org) predicts potential phosphorylations at Tyr-4, Ser-117, and Ser-591, but none of these modifications have been found experimentally.

Regulation of ALOX15: Lundqvist et al. [253] have shown that ALOX15 but not ALOX12 or ALOX15B is increased in ischemic heart tissue compared to non-ischemic heart tissue. The resulting increased production of 15-HETE may thus contribute to the pathogenesis of ischemic heart disease. Both the inflammatory and immune response cytokines IL-4 and IL-13 have been shown to upregulate ALOX15 in lung [254,261] and blood monocytes [248,262,263].

Arachidonate 15-lipoxygenase-2 (ALOX15B)

Overview: Mammalian ALOX15B (15-LOX-B, 15-LOX-2) is a cytosolic protein that becomes membrane associated in the presence of calcium as is common for this family of lipoxygenase proteins [234,249,250]. ALOX15B shows a tissue-specific expression and is found in found mainly in prostate, lung, skin, cornea and is also found in macrophages [264-269].

Substrate and product specificity are less diverse than found for ALOX15. ALOX15B converts arachidonic acid into 15(S)-hydroperoxyeicosatetraenoic acid (15S-HPETE) with no 12S activity (Figure 9) and also converts linoleic acid to 13(S)-hydroperoxyoctadecadienoic acid (13S-HPODE) [232,268,270,271]. ALOX15B oxygenates membrane phospholipids in addition to free fatty acids when in the membrane bound state, following an influx of calcium, and does so at 10 times the rate observed for the cytosolic state [272]. The ALOX15B product 15S-HETE inhibits cell cycle progression in prostate cancer cells [265,266] and marcophages, and serves a pro-inflammatory and pro-atherogenic role in atherosclerotic lesions. Three additional splice isoforms are also produced in humans [265]. Although ALOX15B activity is observed in multiple locations including the cytoplasm, cytoskeleton, cell-cell border, and the nucleus, the three splice isoforms are excluded from the nucleus [266].

Human ALOX15B structure: Human ALOX15B (hALOX15B, UniProtKB-O15296) is a monomeric, non-heme iron protein and a member of the lipoxygenase family. There are four known splice variant isoforms (A-D), all of which have been observed at the protein level [265,266,273]. In addition, there are 3 natural SNP variants (R486H, Q656R, I676V) [22,265,268,274]. hALOX15B isoform A (hALOX15B-A) is a 676 amino acid polypeptide with a calculated molecular weight of 75,857 Da [265]. Isoform B (hALOX15B-B, splice variant a) is missing residues 481-429 and 483-527, isoform C (hALOX15B-C, splice variant b) is missing residues 561-617 and 618-676, and isoform D (hALOX15B-D, splice variant a) is missing residues 401-429. One X-ray structure for isoform A is available (PDB entry 4NRE). There is one potential phosphorylation site (Thr-29) and two potential acetylation sites (Lys-198, Lys-204) on hALOX15B-A, none of which have been confirmed experimentally (PhosphoSitePlus, www.phosphosite.org).

Regulation of ALOX15B: The expression of ALOX15B changes dramatically in various cancers, inflammatory disease and under hypoxic conditions. Ginsberg et al. [275] have shown that the expression of ALOX15B is reduced in aggressive prostate cancer cells as compared to nonaggressive prostate cancer cells. Similarly, Gonzalez et al. [276] report that ALOX15B expression is inversely related to the tumor grade and tumor cell proliferation of lung carcinomas. More specifically, ALOX15B expression in tracheal/bronchial epithelial cells is induced by TNFα and decreased by the TH1 cytokine INFγ, suggesting an active role in mediating airway diseases such as asthma [277]. ALOX15B expression in macrophages under hypoxic conditions is enhanced [278] and the enhancement appears to be mediated by Hypoxia-inducible factor 1-alpha (HIF-1α) [274]. Lastly, exposure of keratinocytes to UV-radiation, known to reduce inflammation in psoriasis patients, results in the enhanced expression of ALOX15B whereas the expression of ALOX12 is reduced [264].

Phosphorylation, glycosylation or other posttranslational modifications involved in regulation or intracellular segregation have not been observed for ALOX15B. However, allosteric regulation of substrate specificity has been reported. ALOX15B converts arachidonic acid and linoleic acid to their respective products, 15S-HPETE and 13S-HPODE. Both Joshi et al. [279] and Wecksler et al. [270] have investigated the effect these hydroperoxy-products have on the substrate specificity and found that the linoleic acid product, 13S-HPODE, binds to the enzyme and allosterically promotes arachidonic acid oxygenation three-fold over linoleic acid oxygenation whereas the arachidonic acid product 15S-HPETE had no effect on the specificity.

Arachidonate 5-lipoxygenase (ALOX5)

Overview: Arachidonate 5-lipoxygenase (ALOX5, 5-LO, 5-lipoxygenase) is a member of the lipoxygenase family of proteins. An excellent review of this enzyme has been presented by Rådmark [241]. ALOX 5 is expressed primarily in cells that are involved in regulating inflammation, allergy, and other immune responses (e.g. granulocytes, monocytes, macrophages, mast cells, dendritic cells, and B-lymphocytes) [280]. It is also strongly expressed in Langerhan cells of the skin [241]. Although ALOX5 persists in the cytosol, upon an increase in intracellular Ca^{2+}, binding of the cation to the PLAT domain of the protein causes it to become associated with the nuclear membrane where it is catalytically active [246,282]. ALOX5 catalyzes the conversion of arachidonic acid to hydroperoxyeicosatetraenoic acid (5-HPETE) which is then rapidly converted to other products (Figure 10A). Release of 5-HPETE to ubiquitous cellular glutathione peroxidases (GPX) results in its reduction to 5-hydroxyeicosatetraenoic acid (5-HETE) [280]. Alternatively, ALOX5 may convert the transient 5-HPETE to its 5,6 epoxide, leukotriene A_4 (LTA_4) [280,283]. Additionally, ALOX5 can convert the ALOX15 product 15-HPETE (Figure 10B) to the epoxide product 5(6)-epoxy-15(S)-hydroxyeicosatetraenoic acid (5-epi-15(S)-HPETE), a precursor to the synthesis of lipoxin A_4 (LXA_4) and lipoxin B_4 (LXB_4) [255-258,284].

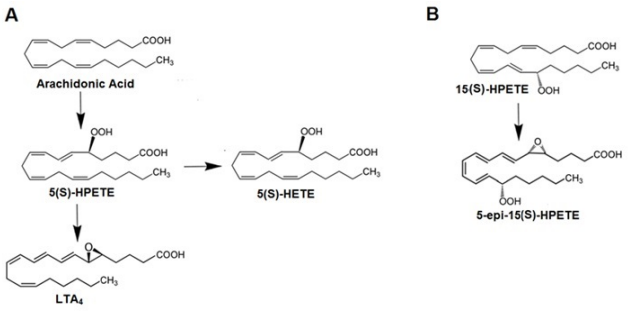

Figure 10: Enzymatic reactions involving ALOX5. A. For arachidonic acid substrate. B. For 15-HPETE substrate.

The mechanism of action of ALOX5 is multistep [283]. Following the release from the nuclear membrane by phospholipase A_2, arachidonic acid binds to the membrane spanning five-lipoxygenase activating protein (FLAP, ALOX5AP) [283,285-288]. Upon an increase in intracellular calcium, ALOX5 is directed to the nuclear membrane where FLAP presents the arachidonic acid to the now membrane-bound ALOX5. At this point the aforementioned reactions commence.

Human ALOX5 structure: Human ALOX5 (hALOX5, UniProtKB-P09917) is a monomeric, non-heme iron protein and a member of the lipoxygenase family. There are six known splice variant isoforms of hALOX5 [280,289-291] and one reported SNP variant (E254K) [23]. Isoform 1 (hALOX5) is the fully biologically competent form. Isoform 2 (Δ13, missing 559-615), isoform 3 (Δ10-13, missing 424-455), isoform 4 (Δ10-13, missing 425-533 and 534-674) and isoform 5 (α10, missing 485-674) have been observed at the protein level [292]. However, none of these additional isoforms are catalytically active, but serve to indirectly regulate the activity of hALOX5. An additional splice variant (5-LOΔ3) has been observed at the mRNA level, but was found to be degraded before translation via the non-sense-mediated mRNA decay system [288]. hALOX5 is a 674 amino acid polypeptide,

reduced to 673 amino acids after removal of the initiator methionine with a final calculated molecular weight of 77,852 Da. Several X-ray structures are available (e.g. PDB entry 3O8Y). There are three known phosphorylation sites, Ser-271, Ser-523, and Ser-663 in the processed polypeptide [293-295,296]. There are also 20 additional potential phosphorylation sites, and one potential acetylation site (Lys-527 in the processed polypeptide) on hALOX5, none of which have been confirmed experimentally (PhosphoSitePlus, www.phosphosite.org).

Regulation of ALOX5: Transforming growth factor β (TGF-β) has been shown to moderately induce ALOX5 activity. However, in combination with 1,25-dihydroxyvitamin D_3, an increase in ALOX5 mRNA and protein is observed in human leukemia 60 cells (HL-60) [297]. The concomitant increase in catalysis, however, far exceeds the increase in ALOX5 protein, in fact, Mono Mac cells treated with both enhancers exhibit a 100-fold increase in expression and a 500-fold increase in activity [298,299]. Coactosin-like protein (CLP) also binds to ALOX5 and in the presence of Ca^{2+} it enhances the activity and stability of ALOX5, even in the absence of membrane or phosphatidylcholine [300,301]. Although both CLP and ALOX5 are cytosolic in the cellular resting state, upon stimulation they co-migrate to the nuclear membrane [301]. Co-expression with either the Δ-13 or Δ-p10 isoforms of ALOX5, serves to reduce the production of LXA_4 and 4-HPETE products up to 44% [292]. It is thought that this reduction in activity is caused by binding of these non-catalytic isoforms to FLAP, thus reducing the availability of this protein to activate hALOX5.

There are three ALOX5 phosphorylation sites that affect catalytic activity [294-296,302,303]. Phosphorylation of Ser-663 by active extracellular signal-regulated kinase-2 (ERK2) results in an increase in ALOX5 catalytic activity and can substitute for Ca^{2+} activation [296,303]. The presence of arachidonic acid, oleic acid, or linoleic acid increases phosphorylation of ALOX5 by ERK2 while stearic acid, palmitic acid and 5-HETE have no effect. Phosphorylation of Ser-271 by mitogen-activated protein kinase-activated protein kinase 2 (MAPKAPK2) also increases the activity of ALOX5 and this phosphorylation markedly enhances the activity in the same manner as observed for ERK2 [297]. This phosphorylation has also been shown to block nuclear export of ALOX5 [298]. On the other hand, phosphorylation of Ser-523 by protein kinase A (PKA) reduces the activity of ALOX5 and at the same time shifts the distribution of the enzyme from the nucleus to the cytoplasm [295,304].

There are reports that suggest arachidonic acid can also cause substrate inhibition [294]. However, Masters et al. [305] have shown that the apparent substrate inactivation is in fact a function of emulsion formation of arachidonic acid leading to a lower solubility and concomitant availability of the substrate.

Arachidonate 12(S)-lipoxygenase (ALOX12)

Overview: Mammalian Arachidonate 12(S)-lipoxygenase (ALOX12,12-LO) is a cytosolic protein that becomes membrane associated, in particular, perinuclear or nuclear membrane sites, in the presence of epidermal growth factor (EGF) [306]. The fact that EGF is known to increase intracellular calcium [307] and that the enzyme structure shows the PLAT calcium binding domain common for this family of lipoxygenase proteins [234, 249, 250], suggests that it is calcium binding that directs this enzyme to the membrane. ALOX12 shows a tissue-specific expression and is found mainly in platelets [308-311], but also in vascular smooth muscle [312], pancreatic cells [313], prostate [314], CNS [231], and skin cells [306]. ALOX12 catalyzes the conversion of arachidonic acid to (12S)-12-hydroperoxy-(5Z,8Z,10E,14Z)-5,8,10,14-eiocosatetraenoic acid (12-HPETE) which is subsequently reduced to (12S)-12-hydoxy-(5Z,8Z,10E, 14Z)-5,8,10,14-eiocosatetraenoic acid (12-HETE), presumably through the action of the ubiquitous GPX (Figure 11) [310, 314, 316]. To a lesser extent, ALOX12 catalyzes the conversion of LTA4 to both lipoxins (5S,6R,15S)-trihydroxy-(7E,9E,11Z,13E)-eicosatetraenoate (LXA_4) and (5S,14R,15S)-trihydroxy-(6E,8Z,10E,12E)-eicosatetraenoate (LXB_4) [243]. ALOX12 is also known to convert (7E, 9E,11Z,14Z)-(5S,6S)-5,6-epoxyicosa-7,9,11,14-tetraenoate and (7E,9E, 11Z,14Z)-(5S,6S)-5,6-epoxyicosa-7,9,11,14-tetraenoate to LXA_4 and LXB_4 respectively [317].

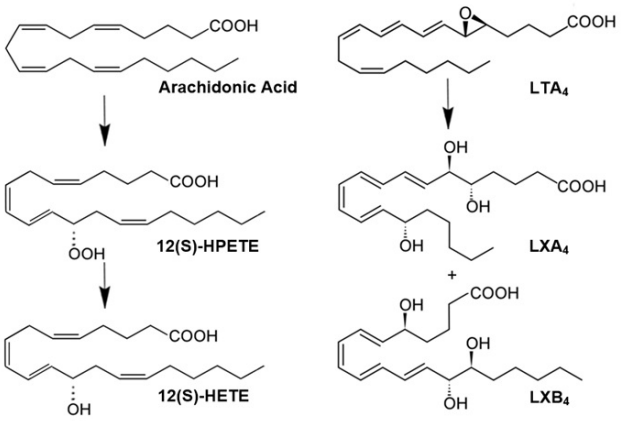

Figure 11: Two of the reactions catalyzed by ALOX12.

Human ALOX12 structure: Human ALOX12 (hALOX12, UniProtKB-P18054) is a monomeric, non-heme iron protein and a member of the lipoxygenase family. There are five known natural SNP variants (E256K, Q261R, A298T, N322S, and R430H) [22,310], but no known splice variant isoforms. hALOX12 is a 663 amino acid polypeptide with a calculated molecular weight of 75,694 Da. There is one X-ray structure available (PDB entry 3D3L).

There are no known posttranslational modifications of ALOX12. However, there are seven potential phosphorylation sites predicted by PhosphositePlus (www.phosphosite.org), one of which, Ser246, corresponds to a known phosphorylation site in the rat counterpart (UniProtKB-F1LQ70 (LOX12_RAT)).

Regulation of ALOX12: IL-1β, IL-4, and IL-8 have been shown to induce ALOX12 mRNA and protein expression in porcine aortic vascular smooth muscle cells (PAVSM) [312]. In addition, PAVSMs treated with high glucose or angiotensin II markedly upregulated both ALOX12 mRNA and protein [318]. Platelet-derived growth factor BB (PDGF) exhibits the same effect on PAVSMs [319]. In human epidermoid carcinoma A431 cells it was found that EGF increases the 12-lipoxygenase mRNA level by about 2-fold with a lag period of 10 hours in parallel with an increase in ALOX12 activity [320]. It has also been observed that nuclear factor kappa-light-chain-enhancer of activated B cells (NFKB/Rel) acts as a transcription factor and suppresses the over-expression of 12-lipoxygenase in humans.

Regulation on the protein level has been observed as well. ALOX12 activity is quite sensitive to the cellular redox conditions. Treatment of enzyme preparations with GSH at levels found in epithelial cell cytosol

(0.1-10 mM) inhibited ALOX12 activity [232]. On the other hand, the reaction product 12-HPETE stimulates its own production by increasing lipoxygenase activity whereas the reduction product 12-HETE does not affect activity [321].

Arachidonate 12(R)-lipoxygenase (ALOX12B)

Overview: Arachidonate 12(R)-lipoxygenase (ALOX12B, 12R-LOX, 12R-lipoxygenase) is a cytosolic protein expressed primarily in the skin but in lesser amounts in the esophagus, stomach, lung, tongue, brain and prostate of mammals (www.ncbi.nlm.nih.gov/gene/242) [322,323]. It is the only known human lipoxygenase able to form an R-isomer [324]. In contrast to the pro-inflammatory role of other lipoxygenases, the primary function of this enzyme is in the regulation, proliferation and differentiation of epithelial cells [325,326]. ALOX12B catalyzes the conversion of arachidonic acid to 12R-HPETE which is rapidly converted to 12R-HETE in the cell (Figure 12) [325,327]. Although it is unknown at this point if the conversion to 12R-HETE is a function of ALOX12B or another enzyme (e.g. glutathione peroxidases, GPX), the fact that 12R-HPETE is a substrate for ALOXE3 and these two enzymes are known to work in conjunction with one another strongly suggests the former [325,328]. ALOX12B catalyzes an additional reaction where O-linoleoyl-ω-hydroxyceramide is oxygenated to 9R-hydroperoxy- linoleoyl-ω-hydroxyceramide, a critical process required for the hydrolysis of the linoleoyl moiety that is in turn required for the formation of the corneocyte lipid envelope in mammalian skin [328].

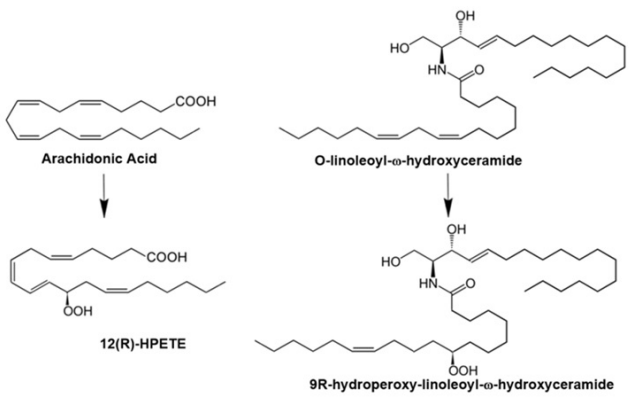

Figure 12: Reactions catalyzed by ALOX12B.

Human ALOX12B structure: Human ALOX12B (hALOX12B, UniProtKB-O75342) is a monomeric, non-heme iron-requiring protein and a member of the lipoxygenase family. There are nineteen known natural SNP variants [329-331], but no known splice variant isoforms. hALOX12B is a 701 amino acid polypeptide with a calculated molecular weight of 80,356 Da. Comparison of the primary sequence to other lipoxygenases shows considerable similarity with the exception of a 31 proline rich amino acid section (e.g. 53.2% with hALOXE3 and 50.4% with hALOX15B without the proline rich region, residues 149-180) that may be involved in regulatory protein-protein interactions [324,327]. A MODBASE protein model was constructed (www.proteinmodelportal.org) that clearly shows this section as a loop in addition to the characteristic PLAT and catalytic domains (data not shown). Sequence comparison indicates that His-398, His-403, His-578, Asn-582, and the carboxyl of the C-terminal Ile coordinate with the catalytic iron. There is one reported X-ray structure (PDB entry 3D3L). There are 11 potential phosphorylation sites on hALOX12B, none of which have been confirmed experimentally (PhosphoSitePlus, www.phosphosite.org).

Regulation of ALOX12B: To date there are no reports on the regulation of ALOX12B expression at the mRNA or regulation at the protein level. There is, however, one report indicating that a specific long non-coding RNA (lncRNA) is required for high mRNA abundance of ALOX12B [332].

Hydroperoxide isomerase (ALOXE3)

Overview: Although ALOXE3 (epidermal LOX-3, e-LOX-3, eLOX-3) is a member of the lipoxygenase family with sequence similarity to both ALOX12B and ALOX15B, its unique catalytic activity places it in its own unique category. ALOXE3 is highly expressed in epidermal tissue [323,333-335] and also found in lower levels in lung, brain, CSF, pituitary, pancreatic islets [228,336] (see also Expression atlas at www.ebi.ac.uk/gxa/genes). One of its biological roles is to isomerize other lipogenase products, in particular HPODEs and HPETEs, to the corresponding epoxy alcohol (hepoxilins) or ketone (Figure 14) [335,337]. ALOXE3 has a wide range of substrate specificities, isomerizing 5-, 8-, 9-, 11-, 12-, and 15-HPETEs as well as 9- and 13-HPODEs. It does exhibit at least a 2:1 preference for the R-hydroperoxy over the S-hydroperoxy substrates (Figure 13) with 12R-HPETE as the best substrate. 12R-HPETE is converted to a 3:2 mixture of 8R-hydroxy-11R,12R-epoxyeicosatrienoic acid (hepoxilins A_3, HxA_3) and 12-ketoeicosatetranoic acid (Figure 14B). A-type hepoxilins are extremely labile and are converted non-enzymatically to the corresponding A-type trioxilin under either weakly acidic or basic conditions. B-type hepoxilins are more stable, but are converted enzymatically to the corresponding B-type trioxilin by one of the ubiquitous soluble epoxide hydrolases [227].

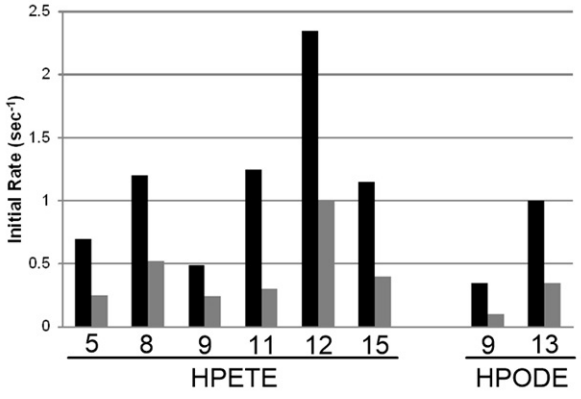

Figure 13: Substrate specificity of human ALOXE3 (eLOX3). Initial rates of reaction for Human recombinant ALOXE3 on different HPETE and HPODE substrates. R configuration hydroperoxides are shown in black bars, S configuration hydroperoxides in grey bars. Rendered from Yu et al. [340].

Another noteworthy reaction involves the isomerization of the product from the ALOX12B catalyzed oxygenation of O-linoleoyl-ω-hydroxyceramide to 9R-hydroperoxy- linoleoyl-ω-hydroxyceramide. ALOX3 converts this product to 9R,10R-trans-epoxy-11E,13R-hydroxy and 9-keto-10E,12Z esters of ceramide.

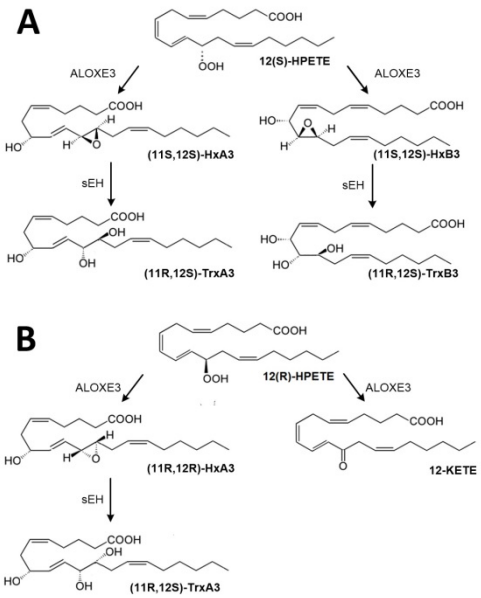

Figure 14: Lipoxygenase catalysis to hepoxilins and trioxilins by ALOXE3. A) Reactions from 12(S)-HPETE substrate. The reactions produce two products, 8Rhydroxy-11S,12S-epoxy-5Z,9E,14Z-eicosatetraenoic acid ((11S,12S)-HxA_3) and 10Rhydroxy-11S,12S-epoxy-5Z,9E,14Z-eicosatetraenoic acid ((11S,12S)-HxB_3). Each of 107 these are converted to the corresponding trioxilins, 8R,11R, 12S-trihydroxy-5Z,9E,14Zeicosatetraenoic acid (11R,12S)-TrXA3) and , 10R,11R,12S-trihydroxy-5Z,9E,14Zeicosatetraenoic acid (11R, 12S)-$TrXB_3$) respectively by specific soluble epoxide hydrolases (sEH). B) Reactions from 12(R)-HPETE substrate. The reactions produce two products, R-hydroxy-11R,12R-epoxy-5Z,9E,14Z-eicosatetraenoic acid (11R,12R)-HxA_3) and 12-oxo-5Z,8Z,10E,14Z-eicosatetraenoic acid (12-KETE). 11R,12R)-HxA_3 can be converted to 8R,11R,12R-trihydroxy-5Z,9E,14Z-eicosatetraenoic acid (11R, 12S)-$TrxB_3$) by sEH. Rendered from Cronin et al. [227], Yu et al. [335], and text within.

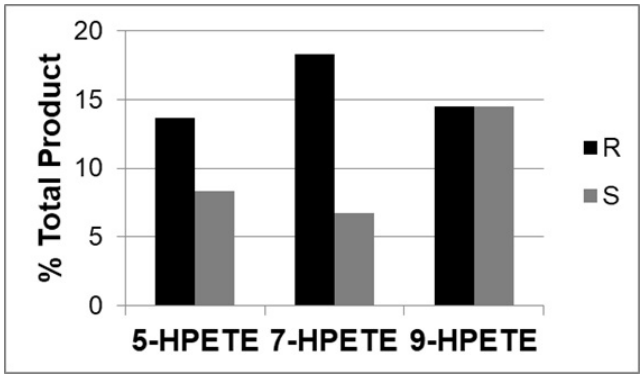

Figure 15: Dioxygenase activity of ALOXE3. Distribution of products produced by ALOXE3 from arachidonic substrate. Rendered from Zheng and Brash [338].

The 9R,10R-transepoxy-11E-13-keto derivative is the most prominent ceramide ester in mouse skin. These reactions are critical processes for the formation of the corneocyte lipid envelope in mammalian skin [328].

Until 2010, reports on the activity of this enzyme indicated that it has no dioxygenase activity. However, in that year Zheng and Brash published two articles [337,338] showing that under specific reaction conditions, ALOXE3 is capable of dioxygenase activity, albeit it much slower than isomerase activity. Under high concentration of hydroperoxide activator and oxygen, the normally long lag phase for dioxygenation can be reduced and arachidonic acid can be converted to a mixture of HPETEs (Figure 15).

Human ALOXE3 Structure: Human ALOXE3 (hALOXE3, UniProtKB-Q9BYJ1) is a monomeric, non-heme iron protein and a member of the lipoxygenase family. It has a sequence similarity to hALOX12B and hALOX15B of 54.1% and 48.4% respectfully and shares similar sequence-derived structural features (catalytic iron, PLAT and alpha-helical domains). There are seven known natural SNP variants (L237M, G281V, QYVA344-347P, R396S, L427P, V500F, P630L) [329,330,339,340], and one known isoform lacking the initiation Met, but there is no confirmation for this isoform at the protein level [77]. hALOXE3 is a 711 amino acid polypeptide with a calculated molecular weight of 80,543 Da. No X-ray crystal structures have been reported for this protein. However, a MODBASE protein model was constructed (data not shown) (www.proteinmodelportal.org) that clearly shows the characteristic PLAT and alpha-helical catalytic domains. Sequence comparison to other lipoxygenases indicates that His-408, His-413, His-588, Asn-592, and the carboxyl of the C-terminal Ile-711 coordinate with the catalytic iron. There are 7 potential phosphorylation sites on ALOXE3, none of which have been confirmed experimentally (PhosphoSitePlus, www.phosphosite.org).

Regulation of ALOXE3: To date there are no reports on the regulation of ALOXE3 expression at the mRNA or protein level. There is one report indicating that a specific long non-coding RNA (lncRNA) is required for high mRNA abundance of ALOXE3 [333].

Leukotriene biosynthesis

Overview: The Leukotriene family of molecules consists of oxygenated products of arachidonic acid of which several are derivatized by glutathione.

The molecules are created through the action of ALOX5 on arachidonic acid, resulting in the transient formation of 5S-HPETE which is then converted by ALOX5 to leukotriene A_4 (LTA_4). The highly unstable LTA_4 can be converted by hydrolysis to leukotriene B_4 (LTB_4) or to leukotriene C_4 (LTC_4) by addition of glutathione (Figures 16 and 17). Stepwise hydrolysis of the peptide portion of attached glutathione on LTC_4 leads to the formation of leukotriene D_4 (LTD_4) and leukotriene E_4 (LTE_4) as shown in Figure 18. LTB_4 is one of the most potent chemotactic molecules known and induces recruitment and activation of monocytes, neutrophils, and eosinophils [341-343]. LTC_4, LTD_4 and LTE_4 are known as the cysteinyl-leukotrienes and are potent bronchoconstrictors, known to increase vascular permeability in postcapillary venules, and known to stimulate mucus secretion [342].

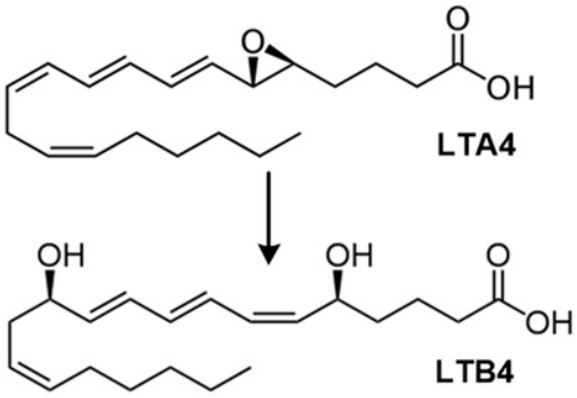

Figure 16: Reaction catalyzed by LTA4H to convert LTA_4 to LTB_4.

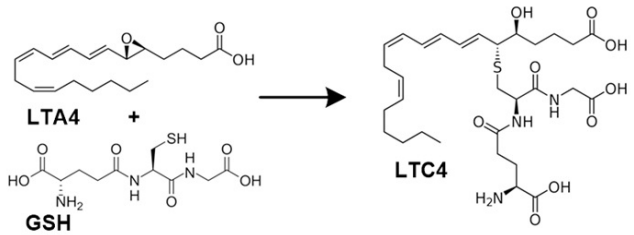

Figure 17: Reaction catalyzed by leukotriene C4 synthase (LTC4S).

Leukotriene A-4 hydrolase (LTA4H)

Overview: Leukotriene A-4 hydrolase (LTA4H, LTA-4 hydrolase) is a cytosolic protein expressed in monocytes, lymphocytes, neutrophils, reticulocytes, platelets, fibroblasts and found in abundance in lung, kidney, spleen, intestines, and reproductive organs [341,344,345]. It is a dual function enzyme catalyzing the conversion of 5S-trans-5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid (Leukotriene A_4, LTA_4) to 5S,12R-dihydroxy-6,14-cis-8,10-trans-eicosatetraenoic acid (Leukotriene B_4, LTB_4), a neutrophil, chemoattractant (Figure 16), and has anion-dependent aminopeptidase activity [344,346,347]. The natural peptide substrates for this enzyme are currently unknown [346].

Human LTA4H structure: Human LTA4H (hLTA4H, UniProtKB-P09960) is a monomeric, zinc-requiring protein and a member of the peptidase M1 family of proteins. hLTA4H is expressed as a 611 amino acid polypeptide and posttranslationally modified to remove the initiator methionine giving it a calculated molecular weight of 69,154 Da [348]. There is one reported natural SNP variant (Y131H), and three reported splice variant isoforms [77,349], none of which have been observed at the protein level. There are numerous reported crystal structures for hLTA4H (e.g. PDB entry 1HS6). Within a narrow otherwise hydrophobic pocket there are three hydrophilic residues Gln-133, Tyr-266, and Asp-374 that are thought to bind LTA_4 [344]. His-295, His-299, and Glu-318 coordinate with the zinc ion to facilitate catalysis.

There are four known lysine acetylation sites on LTA4H, Lys-73, Lys-337, Lys-414 and Lys-573, for which the biological function of each has yet to be determined [99]. One phosphorylation has been reported (see below) at Ser-416. Numerous additional phosphorylation sites are predicted by PhosphoSitePlus (www.phosphosite.org), none of which have been confirmed experimentally.

Regulation of LTA4H: Regulation of LTA4H at the protein level has been reported. Phosphorylation of Ser-416 from a yet-to-be determined kinase inactivates the epoxide hydrolase activity, but not the amino peptidase activity [350]. Treatment of LTA4H with protein phosphatase-1 will restore activity, but only in the presence of an amino peptidase substrate or product.

Both cations and anions reversibly affect the activity of LTA4H. Anions, chloride and thiocyanate in particular reversibly stimulate the peptidase, but not the epoxide hydrolase activity [351]. Maximal chloride stimulation occurs at 100 mM, which is close to the extracellular concentration, suggesting that the peptidase function for LTA4H is primarily extracellular, whereas the epoxide hydrolase function is primarily intracellular. Zinc and other divalent cations reversibly inhibit LTA4H in a dose dependent manner [352]. Although zinc is required for activity, at concentrations higher than a 1:1 enzyme to zinc ion ratio, zinc inhibits the activity, with peptidase activity affected at lower concentrations than the epoxide hydrolase activity.

Orning et al. [353,354] have reported a mechanism-based inactivation of LTA4H by substrate LTA_4 *in vivo* and *in vitro*. The inactivation involves a 1:1 covalent binding of the substrate to the catalytic site which inhibits both the peptidase and hydrolase activities. They further propose that the hydrolase reaction is capable of two pathways, one leading to inhibition and the other to product LTB_4, the former eventually leading to 100% inactivation.

Leukotriene C4 synthase (LTC4S)

Overview: Leukotriene C4 synthase (LTC4S, LTC_4 synthase) is a transmembrane protein expressed in a limited number of cell types and is found in mast cells, eosinophils, basophils and monocytes [355,356]. The enzyme catalyzes the conjugation of GSH to the unstable LTA_4 produced by ALOX5 [356], producing leukotriene C_4 (LTC_4) (Figure 17), a powerful mediator in pathophysiological conditions such as immediate hypersensitivity and inflammation [357,358]. It is found in the outer nuclear membrane and peripheral endoplasmic reticulum, but not in the inner nuclear membrane [359].

Human LTC4S structure: Human LTC4S (hLTC4S, UniProtKB-Q16873) was originally reported as a homodimer, based on gel filtration data [356,360], but later crystallographic data shows it to be a homotrimer [355,361] and a member of the MAPEG family of proteins. hLTC4S is expressed as a 150 amino acid protein with a calculated molecular weight of 16,567 Da [362]. The crystal structure (e.g. PDB entry 2UUH) shows that each monomer of the biologically functional trimer consists of five alpha helices, four of which are transmembrane and one that extends out of the membrane [355,361]. There is one reported natural SNP variant, R142Q, which is only observed at the cDNA level [22].

Currently, the only confirmed posttranslational modifications are the phosphorylations (see below) at Ser-36 and Thr-40 [363]. Additional phosphorylations at either or both Ser-28 and Ser-111 have also been reported [364].

Regulation of LTC4S: Regulation of LTC4S at the protein level has been demonstrated. Phosphorylation of Ser-36 by p70S6k, a serine/threonine-specific kinase, suppresses the activity of LTC4S [363]. Ser-36 is located on a loop region near the substrate binding site and molecular dynamic simulations reveal that this loop moves to allow hydrogen bonding between the phosphorylated Ser-36 and the catalytic Arg-104 on a neighboring subunit, thus impairing the activity of the adjacent subunit. Phosphorylation of Thr-40 by the same kinase occurs less often and has no effect of the activity of the enzyme. Additional phosphorylations by phosphokinase C (PKC) in THP-1 cells have been reported to reduce the activity of LTC4S [364]. There are two PKC consensus sites on LTC4S involving Ser-28 and Ser-111. It is not known which or if both sites are phosphorylated. In addition, it was found that transfection of recombinant LTC4S into both COS-7 and K-562 cells did not produce phosphorylated LTC4S, indicating that cell-specific effectors are required for phosphorylation.

Regulation at the transcriptional level has also been reported. TGF-β-1, -2, and -3 significantly increase the production of LTC4S mRNA in THP-1 cells and have no effect on the half-life of the mRNA [365]. Exposure of THP-1 cells to LPS, a known mediator of inflammatory response at biologically relevant levels, results in the down-regulation of LTC4S mRNA [366]. The biological consequence of this action is unclear at this time.

Synthesis of Leukotriene D_4 (LTD_4) and Leukotriene E_4 (LTE_4)

Overview: The enzymes involved in the production of leukotriene D_4 (LTD_4) and leukotriene E_4 (LTE_4) (Figure 18) are involved in numerous biological processes other than leukotriene biosynthesis. Only the production of LTD_4 and LTE_4 are discussed here.

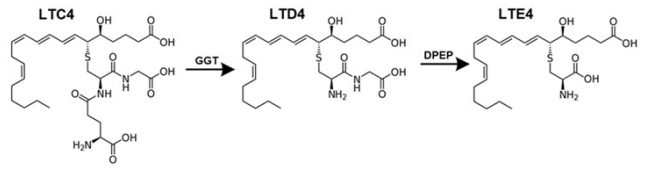

Figure 18: Synthesis reactions for LTD_4 and LTE_4.

Gamma-glutamyl transaminase (GGT)

Overview: Gamma-glutamyl transaminase (GGT1) catalyzes the transfer of the γ-glutamyl group from glutathione and related compounds, such as LTC_4 to an array of amino acids and peptides or glutathione itself, playing a key role in glutathione metabolism [367]. Its activity has been observed in kidney, pancreas, epidermis, seminal vesicles, liver, spleen cells, bile, seminal fluid, blood serum, and urine. It is found in the largest amounts in mammalian kidneys.

Human GGT structure: Human Gamma-glutamyl transaminase (hGGT1, UniProt-P19440) is a glycosylated heterodimer and member of the N-terminal nucleophile superfamily of proteins [368]. The original transcript is 569 residues, but is autocleaved between Gly-380 and Thr-381 to produce a heavy chain (1-380) and a light chain (381-569), thus forming the heterodimer [369]. The protein is localized to the plasma membrane by a single pass helix located on the N-terminus of the large subunit. The crystal structure (e.g. PDB entry 4GDX) shows a stacked α–β–β–α core similar to other family members. The N-terminal Thr-361 of the light chain provides the nucleophilic active site residue [368]. There are three known isoforms of this protein [370,371]. Isoform 2 (341-366: VVRNMTSEFFAAQLRAQISDDTTHPI → ASSGVSAGGPQHDLRVLRCPAPGPDL and 367-569: Missing) is an alternate splice isoform thus far only identified at the mRNA level. Isoform 3 (1-344: Missing) is produced by alternative promotor usage and has been observed at both the mRNA and protein level [370]. The function of this protein isoform is unknown at this time.

There are seven potential glycosylation sites on hGGT1, all of which have been confirmed experimentally [372,373]; Asn-95, Asn-120, Asn-230, Asn-266, Asn-297, Asn-344, an Asn-511 are confirmed. Not all of the glycosylation sites are modified at the same time and different N-glycans may be attached to the same residue in different molecules [373]. There is one predicted phosphorylation site at Thr-550 which is not confirmed experimentally (PhosphoSitePlus, www.phosphosite.org).

Regulation of hGGT1: Transcription of the gene for hGGT1 is not highly inducible. Agents such as ethanol and steroids only cause a two to five-fold change in expression and many other xenobiotics have no effect [374,375]. However, the regulation of this gene is complex. There are at least seven promoters, although levels of GGT expression in individual organs seem to be relatively constant [376]. In addition, the expression of the different types is tissue dependent. For example, there are six types of GGT expressed in the kidney, but only one type expressed in the intestine. This is perhaps a reflection of the vast number of different processes this enzyme participates in.

Dipeptidase (DPEP)

Overview: Dipeptidase (DPEP, RDP, MDP) hydrolyzes a wide range of dipeptides, including the renal metabolism of glutathione and its conjugates, beta-lactam rings, as well as the conversion of LTD_4 to LTE_4 [377-380]. DPEP activity has been observed in lung, kidney, pancreas and testis, spleen, liver, serum and heart, and intestine [381].

Human DPEP structure: Human dipeptidase (hDPEP1, UniProtKB-P16444) is a glycosylated, zinc-requiring homodimer with subunits of 59 kDa each and is a member of the Peptidase M19 family [382]. The protein is plasma membrane associated through a glycosyl-phosphatidylinositol anchor [383]. The originally transcribed polypeptide consists of 411 amino acid residues. Only 1 protein isoform has been identified, however, three natural SNP variants have been reported (R246H, E351K, and E351Q) [204]. Residues 1-16 are removed as a signal peptide and residues 386-411 are removed to activate the enzyme [384,385]. The resulting protein consists of 369 amino acids with a calculated molecular weight of 41,062 Da and is covalently attached to an identical monomer through a disulfide bond (Cys-377, Cys 361 in the processed polypeptide) [380,384]. The crystal structure (e.g. PDB entry 1ITQ) shows that each monomer of the biologically functional dimer contains a catalytic binuclear zinc center coordinated by His-36, Asp-38, His-214, His-235, and a bridging water and Glu-141 [380, 386]. The overall structure of each monomer is a distorted $(\alpha/\beta)_8$-barrel fold [386].

There are four potential glycosylation sites on hDPEP1, three of which have been confirmed experimentally; Asn-57, Asn-279, Asn-332 are confirmed and Asn-358 is a potential site [58,380]. There is also a glycosyl-phosphatidylinositol attached to the processed C-terminal serine that serves to anchor each subunit to the membrane [385].

There are 10 potential phosphorylation sites, none of which have been confirmed experimentally (PhosphoSitePlus, www.phosphosite.org).

Regulation of hDPEP1: It has been reported that DPEP1 is upregulated in colorectal cancers and expression levels are associated with positive lymph node metastasis. The particular mechanisms involved have yet to be discovered [387].

Eoxins Biosynthesis: EXA_4, EXC_4, EXD_4 and EXE_4

Overview: In effect, eoxins are the C14,15 oxidized isomers of leukotrienes and are produced from arachidonic acid via ALOX15 to eoxin A_4 (EXA_4) and then converted through a linear path to eoxin C_4 (EXC_4) , eoxin D_4 (EXD_4) and eoxin E_4 (EXE_4) by the same enzymes used for the production of leukotrienes (Figure 7). For this reason this pathway will not be discussed in detail.

Eoxins are pro-inflammatory metabolites of arachidonic acid and are produced in cells that express significant amounts of ALOX15, human airway epithelial cells, eosinophils, subsets of mast cells, mast cells and dendritic cells [388]. Their biological roles have yet to be thoroughly explored; however, it is known that they serve to increase vascular permeability [388].

The enzymes involved in the production of EXA_4, EXC_4, EXD_4, and EXE_4, are involved in the synthesis of other eicosanoid previously discussed. Figure 19 outlines the proposed pathway for eoxin biosynthesis [11,388-390]. The pathway begins with the conversion of arachidonic acid to 15-HPETE by ALOX15 or ALOX15B. This metabolite can be further metabolized by ALOX15 to the 14,15-epoxy equivalent of LTA_4 (14,15-LTA_4) known as EXA_4 to avoid confusion with leukotrienes. At the point LTC4S adds a glutathione residue to EXA_4 to produce EXC_4 and the peptide moiety modified by the subsequent actions of GGT1 and DPEP to produce EXD_4 and EXE_4 respectively.

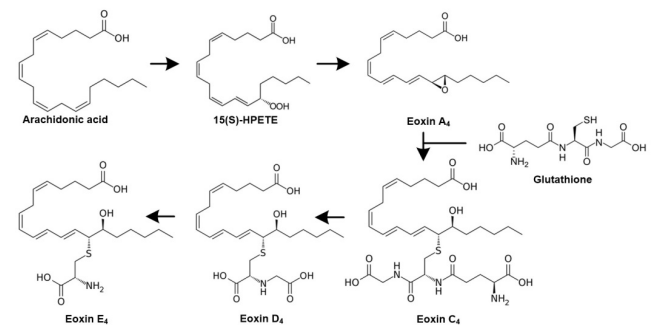

Figure 19: Proposed metabolic pathway for the formation of eoxins. Rendered from Feltenmark et al. [38].

Future Directions

Although much is known about the enzymes of the eicosanoid pathway, there are several areas requiring further study. While preparing this manuscript it became clear that the regulation of these enzymes at the protein level is lacking in detail. Confirming the presence or absence of phosphate at potential phosphorylation sites and the condition under which the modification is made, and the effect of the modification has on the catalytic activity requires further investigation. Confirmation of the kinases involved in all phosphorylations and an examination of the connection between pathways that employ the same kinases is required for a broader understanding of the context for the phosphorylation. A more complete analysis of glycosylation is also in order, including location on the polypeptide, structures, and context dependency of modifications to the glyco-portion (e.g. β-trace), and associated intracellular expression and changes in thereof. Lastly, X-ray crystallographic structures for all eicosanoid enzymes would be most useful.

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