1. INTRODUCTION

1.1 THE ARACHIDONIC ACID CASCADE

Arachidonic acid is one of the major polyenoic fatty acids in mammals and is enriched in the sn-2 position of phospholipids in membranes. It is the precursor of an important group of biologically active compounds, the eicosanoids. Eicosanoids are formed via the arachidonic acid cascade. The first step in the arachidonic acid cascade is the release of AA from phospholipids by the lipid-cleaving enzyme phospholipase A_2. Mammalian cells contain several structurally different phospholipase A_2 (PLA_2) enzymes, emphasising the importance of fatty acid turnover and ensuring specificity of regulatory pathways.

Eicosanoid is the collective name for C_{20} unsaturated lipids derived from arachidonic acid (AA, $C_{20:4}$). The eicosanoids are formed by three different routes; namely via the cyclooxygenase (COX), cytochrome *P*-450 (cyt *P*-450) or lipoxygenase (LOX) pathways. The three pathways are included in the classical arachidonic acid cascade as illustrated in Fig. 1.

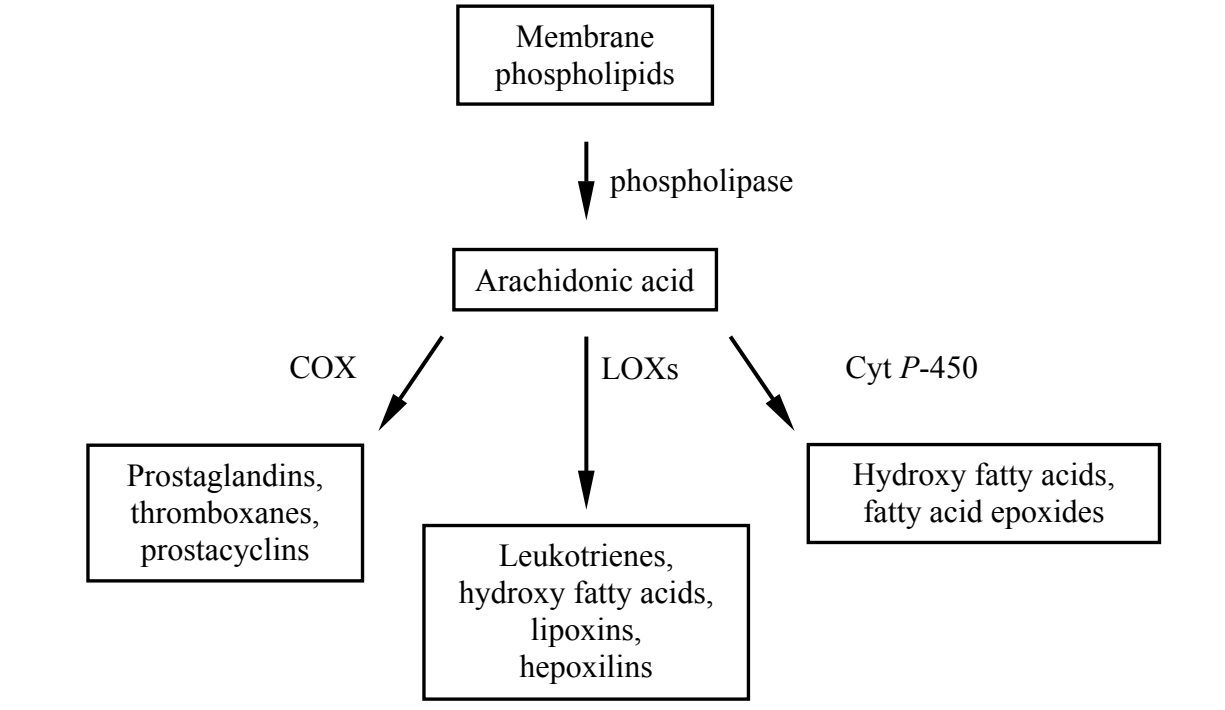

Figure 1. The Classical Arachidonic Acid Cascade

Two main factors regulate eicosanoid syntheses, (i) the availability of substrate and (ii) the activation state of the oxygenases. Either calcium and/or the hydroperoxide tone may control the latter. The 5-LOX requires calcium for translocation of the enzyme to the nuclear

membrane (Rouzer and Samuelsson, 1987) and also requires an activating protein for its regulation (Rouzer *et al*., 1985). The activity is dependent on both calcium and the hydroperoxide tone (Ochi *et al*., 1983; Weitzel and Wendel, 1993). The 15-LOX requires calcium for the translocation of the enzyme to membranes, however, the activity is calcium-independent (Watson and Doherty, 1994; Brinckmann *et al*., 1998; Hoffman et al., 1988). The 15-LOX activity is dependent on the hydroperoxide tone (Vanderhoek *et al*., 1982). The 'threshold peroxide tone' is dependent on the fatty acid concentration present. At high substrate levels 15-LOX is blocked easier by glutathione/GPx. The 12-LOX is calcium-independent, however, requires an increased hydroperoxide tone for the activity (Vanderhoek *et al*., 1982; Walstra *et al*., 1987a).

Many eicosanoids exhibit biological activity and have been shown to possess potent pharmacological activities that may be of physiological or pathological importance. Some of these effects are summarised in Table 1.

1.2 THE LIPOXYGENASES

The lipoxygenases are dioxygenase enzymes derived from a multi-gene family which catalyse the dioxygenation of polyunsaturated fatty acids to their corresponding hydroperoxy derivatives (Yamamoto *et al*., 1997). These products, referred to as hydroperoxyeicosatetraenoic acids (HpETEs), are subsequently converted to the corresponding hydroxyeicosatetraenoic acids (HETEs) by enzymatic or non-enzymatic processes.

1.2.1 Classification of the Lipoxygenases

The lipoxygenases are classified according to their positional specificity of AA oxygenation and in mammalian cells with this respect four types of LOXs have been identified:-

i) 5-LOX - introduces molecular oxygen at position C-5 in the AA backbone resulting in (6*E*,8*Z*,11*Z*,14*Z*)-5*S*-hydroperoxyeicosa-6,8,11,14-tetraenoic acid (5*S*-HpETE).

ii) 12-LOX - introduces molecular oxygen at position C-12 in the AA backbone resulting in (5*Z*,8*Z*,10*E*,14*Z*)-12*S*-hydroperoxyeicosa-5,8,10,14-tetraenoic acid (12*S*-HpETE).

iii) 15-LOX - introduces molecular oxygen at position C-15 in the AA backbone resulting in (5*Z*,8*Z*,11*Z*,13*E*)-15*S*-hydroperoxyeicosa-5,8,11,13-tetraenoic acid (15*S*-HpETE).

iv) 8-LOX - an inducible enzyme reported in mouse skin which introduces a molecular

oxygen at position C-8 in the AA backbone resulting in (5*Z*,9*E*,11*Z*,15*Z*)-(8*S*)-hydroxyeicosa-5,9,11,15-tetraenoic acid (8*S*-HpETE) (Fürstenberger *et al*., 1991, Hughes and Brash, 1991).

Table 1. Physiological/Pathological activities of eicosanoids

Eicosanoid	Activity	Reference
Thromboxane A_2	Platelet aggregation	Hamberg *et al*., 1974, 1975
	vasoconstriction	Hamberg *et al*., 1975
	stimulate coronary arteries	Svensson and Hamberg, 1976
Prostaglandins $F_2\alpha$	proinflammatory	Moncada *et al*., 1973
Prostaglandin H_2	stimulate coronary arteries	Svensson and Hamberg, 1976
Prostaglandin E_2	vasodepressor effects	Armstrong *et al*., 1976
Leukotriene B_4	Chemotaxis, aggregation	Ford-Hutchinson *et al*., 1980
	cell proliferation	Gualde et al., 1985
	PPARα activation	Devchand *et al*., 1996
Leukotriene C	contraction	Murphy et al., 1979
Leukotriene D	contraction	Orning *et al*., 1980
Lipoxin A_4/B_4	monocyte migration and adhesion	Maddox and Serhan, 1996
	vasodilatory	Badr *et al.*, 1989
	vascular permeability	Serhan *et al*., 1999
Hepoxilin A_3	insulin release	Pace-Asciak and Martin, 1984
	second messenger release	Nigam *et al*., 1990b
12-HETE	PI3-kinase activation	Szekeres *et al*., 2000
	angiogenesis and tumour growth	Nie *et al*., 1998
15-HETE	Induced in airway disease	Shannon *et al*., 1993
	Pregnancy-induced hypertension	Mitchell and Koenig, 1991

Several of the mammalian 5-, 8-, 12- and 15-LOXs have been isolated, purified and cloned (Matsumoto *et al*., 1988; Sigal *et al*., 1988; Funk *et al*., 1990; Izumi *et al*., 1990; Yoshimoto *et al*., 1990a,b; Jisaka *et al*., 1997).

The classification system based on positional specificity has several inherent problems which became more evident as more LOXs were isolated and characterised (for review see Kühn and Borngräber, 1999). Thus the positional specificity of LOXs is not an absolute property,

and the specificity is rather substrate related. For instance, the 15-LOX oxidises linoleic acid to 13-HpODE, but it also exhibits 5-LOX activity with the 15-HETE methyl ester as substrate. Similarly, among the arachidonate 12-LOXs, a leukocyte-type 12-LOX has been identified which is similar to the reticulocyte 15-LOX with regards to enzymatic and protein-chemical structures (Yamamoto, 1992). The platelet-type 12-LOX differs from the leukocyte-type with regards to substrate specificity. A similar sub-classification may also be carried out for the 15-LOXs (Kühn and Borngräber, 1999). To-date 15-LOXs have been identified in rabbit reticulocytes (Type I) (Schewe *et al*., 1975) and human skin (Type II) (Brash *et al*., 1997). The two enzymes share only 40% amino acid homology and may not be functionally related. The murine 8S-LOX on the other hand shares 78% homology, at both the DNA and protein level, to Type II 15-LOX (Brash *et al*., 1999). The heterogeneity of the LOX pathways is shown in Fig. 2.

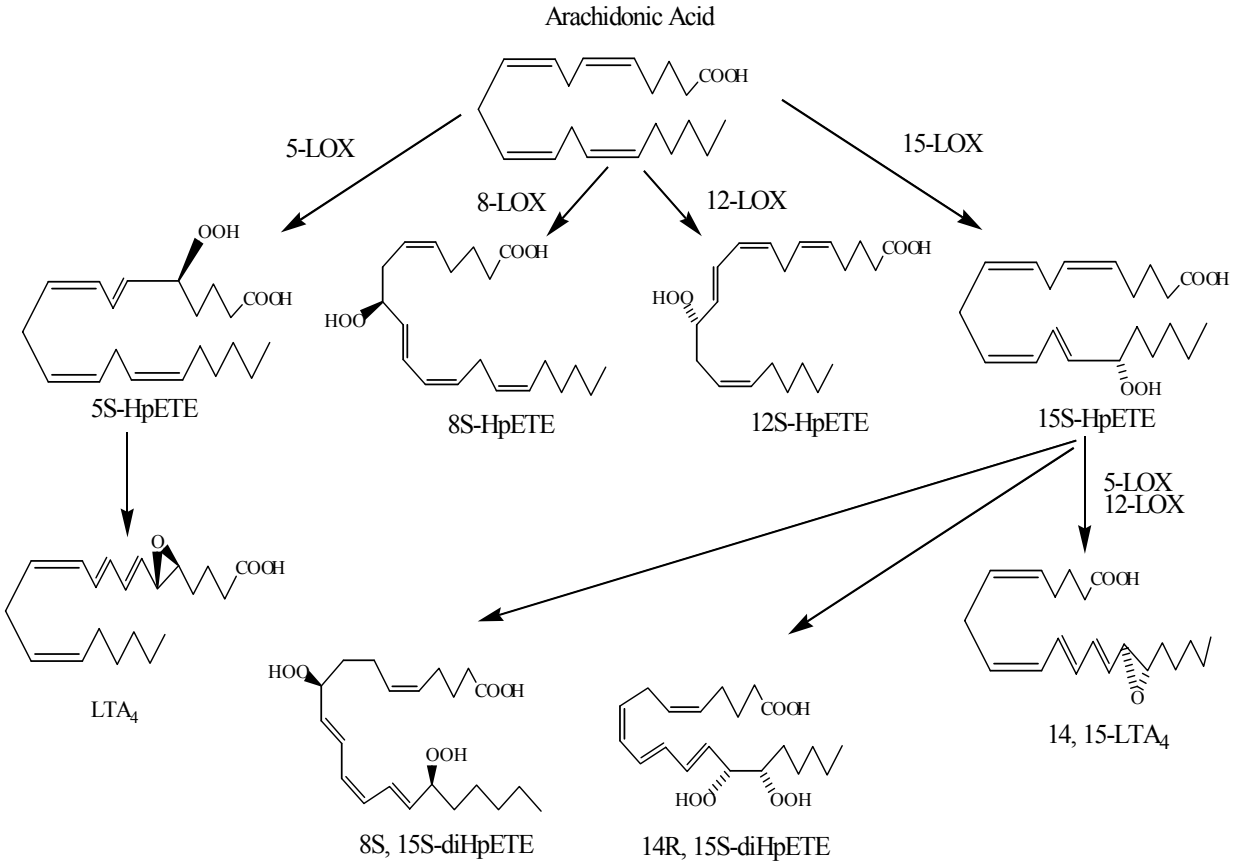

Figure 2. The primary products of the LOX pathways

A phylogenetic tree (Kühn and Thiele, 1999; Brash, 1999) of the mammalian lipoxygenases reveals that the enzymes may be sub-divided into four groups:- (i) the 12S/15S-LOXs

(includes the reticulocyte- and leukocyte-type 12- and 15-LOXs), (ii) platelet-type 12S-LOXs, (iii) 5S-LOXs, and (iv) the epidermis-type LOXs (includes 8S-, 12R and 15S-epidermis-type LOXs) (Fig. 3). The enzymes, however, may differ from other members of the same family with respect to enzymatic properties, i.e. substrate specificity and reactivity with complex lipid-protein assemblies (Kühn and Thiele, 1999).

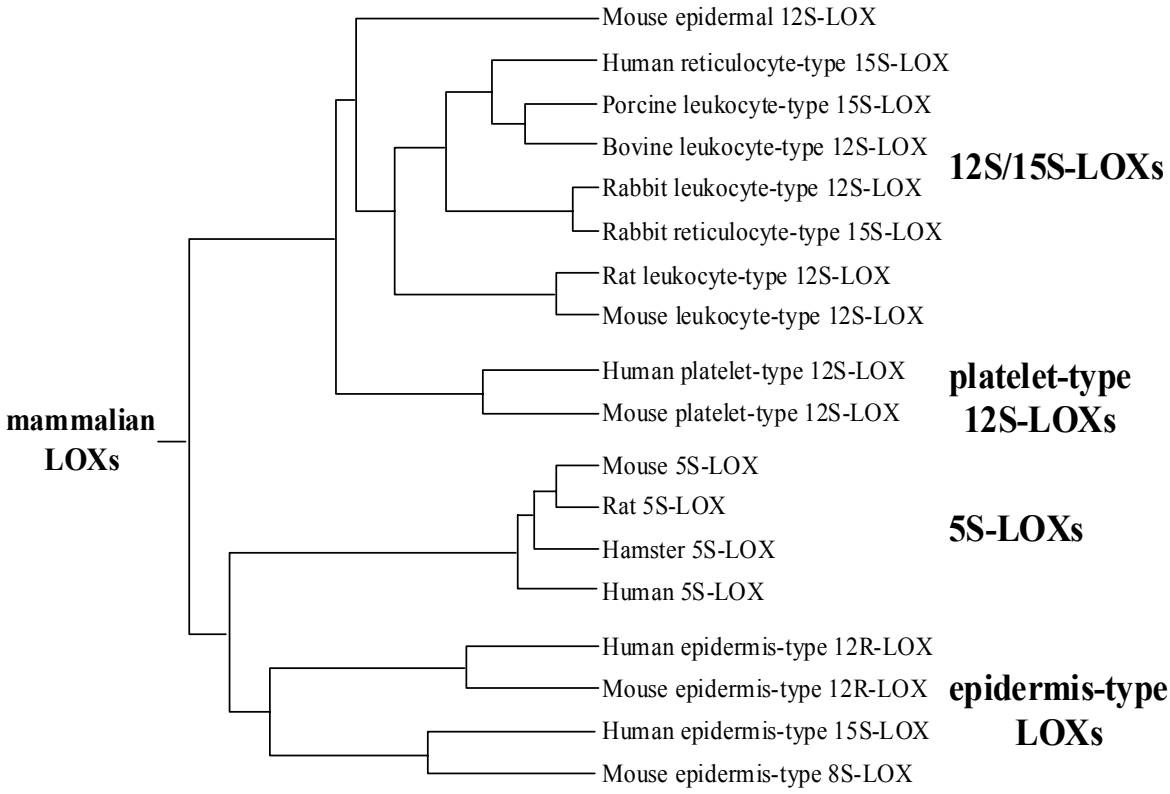

Figure 3. Phylogenetic tree of the mammalian lipoxygenases

(From Kühn and Thiele, 1999)

1.3 THE 12-LIPOXYGENASE PATHWAY

1.3.1 Heterogeneity of the 12-Lipoxygenases

So far at least three 12-LOX types have been characterised from various sources including rat brain (Watanabe *et al*., 1993), porcine leukocytes (Yoshimoto *et al*., 1982, 1990a), bovine epithelium (Hansbrough *et al.,* 1989, 1990), human leukocytes (Yoshimoto *et al*., 1990b), human platelets (Nugteren, 1975) and murine epidermis (Chen *et al*., 1994; Freire-Moar *et al*., 1995). The enzymes exhibit from 60% to 79% homology at the amino acid level as compared to the rabbit leukocyte type 12-LOX (Fig. 4). These enzymes may be differentiated on the basis of a number of factors, including primary structures, substrate specificities, product profiles and suicide inactivation.

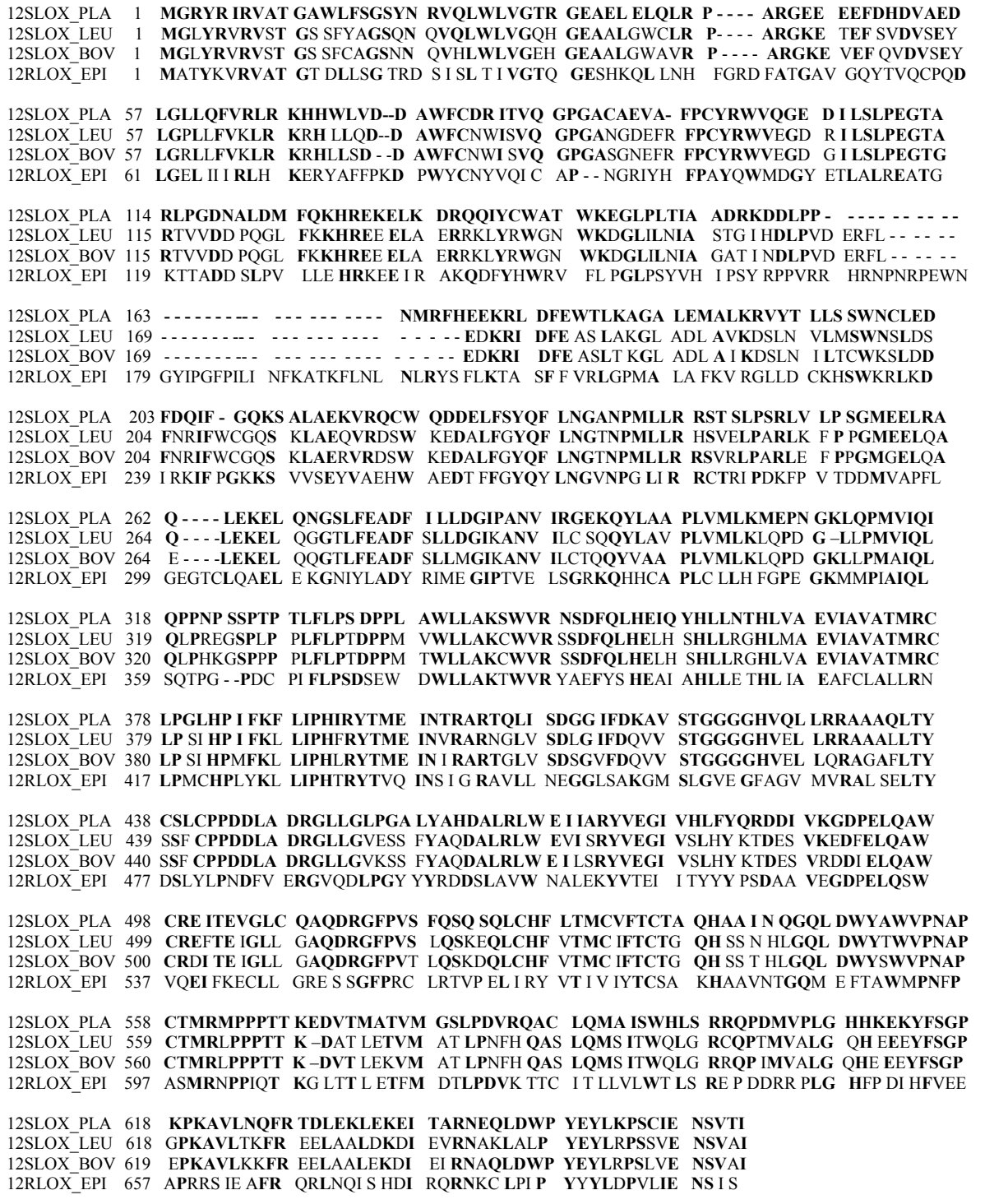

Figure 4. Amino acid sequence alignment of 12-LOX. Sequences sharing identical amino acids with human platelet-type 12-LOX (12SLOX_PLA) are indicated in bold. 12SLOX_LEU = leukocyte-type 12S-LOX, 12SLOX_BOV = bovine tracheal 12S-LOX and 12RLOX_EPI = human skin 12R-LOX.

1.3.2 Platelet-type vs. Leukocyte-type 12(S)-LOX

The leukocyte-type and platelet-type 12-LOXs have been characterised in more detail and can be distinguished as summarised in Table 2. The leukocyte- and platelet-type 12-LOXs were first isolated from porcine leukocytes and human platelets respectively (Yokoyama *et al*., 1985, 1986; Hamberg and Samuelsson, 1974). They have also been found in numerous other animal cells and tissues and plants.

Table 2. 12S-Lipoxygenase Isozymes

	Leukocyte-type	Platelet-type
Substrate Specificity		
Phospholipids	Active	Almost inactive
C18-fatty acids	Active	Almost inactive
C20-fatty acids	Active	Active
Amino acid homology with 15-LOX	75-86%	63-65%
Distribution		
Human	Adrenal cells, breast	Platelet, skin
Porcine	Leukocyte, pituitary	-
Bovine	Leukocyte, trachea, cornea	Platelet
Suicide inactivation	+	-

(Modified from Pace-Asciak, 1994)

1.3.2.1 PRIMARY STRUCTURE AND MULTIFUNCTIONAL CATALYSIS

The leukocyte-type shows 86% amino acid homology with human 15-LOX whereas the platelet-type shows only 63-65% homology (Yoshimoto and Yamamoto, 1995; Yamamoto *et al*., 1997). Whereas the platelet 12-LOX oxygenates arachidonic acid at position C-12 to yield 12-HpETE, the porcine leukocyte-type 12-LOX possesses a second catalytic activity for the position C-15 of AA (Takahashi *et al*., 1988; Kishimoto *et al*., 1996), which gives rise to 15-HpETE. Thus the porcine leukocyte-type 12-LOX produces 15-HpETE equivalent to about 10% of the total products whereas the human platelet-type produces 15-HpETE equivalent to only 1-2% of 12-HpETE.

1.3.2.2 SUBSTRATE SPECIFICITY

The isozymes may also be distinguished by their substrate specificity (Hada *et al*., 1991; Takahashi *et al*., 1988). The platelet 12-LOX almost exclusively utilises C20:4 (AA) whereas the leukocyte 12-LOX utilises a variety of C18 and C20 acids such as linoleic, linolenic and arachidonic acid as substrate. This difference is also evident between the bovine leukocyte- and platelet-type 12-LOX (Takahashi *et al*., 1988). The enzymes also differ in their product profiles when incubated with AA and analysed by HPLC. For example, the murine leukocyte-type produces relatively large amounts of 15S-HETE together with 12S-HETE and is also referred to as 12/15-LOX (Chen *et al*., 1994), whereas the murine epidermis-type is unique, in that it produces 12R-HETE and is referred to as 12R-LOX (Boeglin *et al*., 1998).

1.3.2.3 SUICIDE INACTIVATION

When lipoxygenases are allowed to react with arachidonic acid, the reaction slows down and does not start again by the addition of arachidonic acid (Kishimoto *et al*., 1996). This property is referred to as suicide inactivation, and the effect of which may be used to distinguish between the two enzymes. The porcine leukocyte-type 12-LOX undergoes suicide inactivation within a few minutes when AA is used as substrate. Human platelet-type 12-LOX takes much longer for suicide inactivation (Yokoyama *et al*., 1986; Hada *et al*., 1991; Kishimoto *et al*., 1996). The suicide inactivation of the leukocyte-type 12-LOX is not pronounced when linoleic acid is used as substrate, because this inactivation by AA has been found primarily to be due to the production of 15-HpETE, which reacts about 10 times faster with the leukocyte-type 12-LOX as compared to the platelet-type 12-LOX (Kishimoto *et al*., 1996).

Besides the differences between the leukocyte-type and platelet-type 12-LOXs, differences have also been shown between 12-LOXs from porcine leukocyte, bovine leukocyte and bovine trachea with respect to substrate specificity (Yokoyama *et al*., 1986; Walstra *et al*., 1987a,b; Hansbrough *et al*., 1990). Stereoisozymes of 12-LOX also exist in plants and marine invertebrates. Thus in plants only products of the ´S´ stereochemistry have been found, whereas in marine invertebrates and mammals both ´S´ and ´R´-stereospecific lipoxygenases have been reported (Boeglin *et al*., 1998; Brash *et al*., 1999).

The various 12-LOXs and their heterogeneity have been well characterised and summarised in a number of reviews (Yoshimoto and Yamamoto, 1995; Nakamura *et al*., 1996; Yamamoto *et al*., 1997; 1999).

1.3.3 The Bifurcated Nature of the 12-LOX pathway

The initial product formed by the action of 12-LOX on arachidonic acid is 12-HpETE (Hamberg and Samuelsson, 1974). The 12-LOX pathway is bifurcated at the level of 12-HpETE (Fig. 5), the reductase pathway resulting in 12-HETE and the isomerase pathway leading to the hepoxilins A_3 and B_3 (Bryant and Bailey, 1979; Pace-Asciak *et al*., 1983a, b, c). Both metabolic routes compete with each other for the common substrate 12-HpETE, the concentration of which is determined by the hydroperoxide tone (Bryant *et al*., 1982).

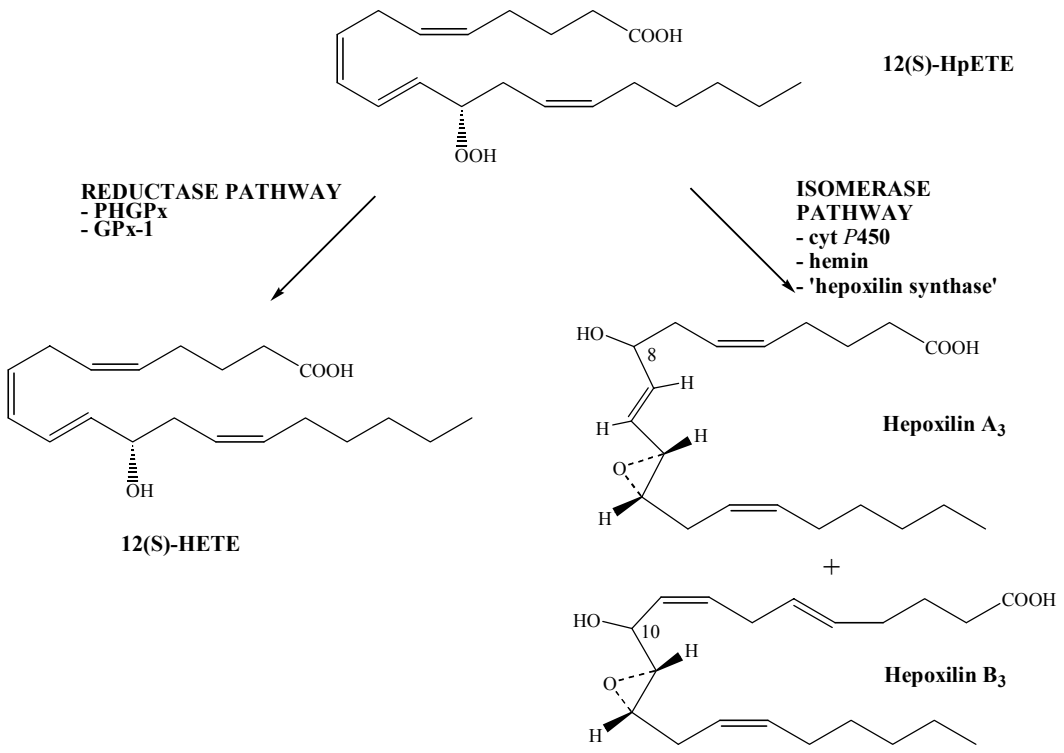

Figure 5. Bifurcated 12-LOX Pathway

1.4 ROLE OF OXIDATIVE STRESS AND THE HYDROPEROXIDE TONE IN EICOSANOID METABOLISM

The hydroperoxide tone can be defined as the steady-state between hydroperoxide generating systems, e.g. cyclooxygenase and LOX reactions, and the hydroperoxide scavenging systems, e.g. GPx-1, PHGPx and glutathione S-transferases. In most mammalian cells the steady-state concentration of hydroperoxides is very low, and therefore mainly hydroxy fatty acids are detected.

The first evidence for the role of the hydroperoxide tone in eicosanoid metabolism was observed in 1979 by Hemler and co-workers. Eicosanoid metabolism depends on the activation of the oxygenases which is dependent on the hydroperoxide tone. Hemler *et al*. (1979) showed that lipid peroxides trigger prostaglandin synthesis and that the synthesis was inhibited dose-dependently by the addition of glutathione peroxidase. Since 1979, plenty of evidence has accumulated which illustrates that the glutathione peroxidases are intimately integrated in the regulation of arachidonic acid metabolism in mammalian cells (Lands *et al*., 1985; Bryant *et al*., 1982, 1983; Weitzel and Wendel, 1993; Schnurr *et al*., 1996; Imai *et al*., 1998). Schnurr *et al*. (1999a, b) found an inverse regulation of the 12/15-lipoxygenase and PHGPx by cytokines.

The enzymes prostaglandin endoperoxide synthase (Lands *et al*., 1985; Marshall *et al*., 1987; Hecker *et al*., 1991) and the lipoxygenases (Yokoyama *et al*., 1986; Ludwig *et al*., 1987) are triggered at low hydroperoxy-polyenoic fatty acid concentrations. At higher concentrations, suicide inactivation and the hydroperoxidase activities of these enzymes are promoted (Härtel *et al*., 1982). Under lipohydroperoxidase activity is understood the capacity of the enzyme to utilise hydroperoxy polyenoic fatty acids as substrate. It is based on the homolytic cleavage of the hydroperoxo binding leading to formation of alkyloxy radical, which is transformed to secondary products, such as hydroxyepoxy derivatives, oxodienes, fatty acid dimers and alkanes.

Oxidative stress is due to an imbalance between the production of reactive oxygen species (ROS); e.g. hydrogen peroxide, lipid hydroperoxides, OH radicals, singlet oxygen and superoxide anions, and the cellular antioxidant defence mechanisms (Sies, 1989, 1997). The ROS are formed by the enzymatic or nonenzymatic oxidation of polyunsaturated fatty acids resulting in hydroperoxy-polyenoic fatty acids. The intracellular defence system against oxidative stress by hydroperoxy-polyenoic fatty acids involves their reduction by enzymes such as GPx-1 and PHGPx (Ursini *et al*., 1982) or the non-selenium glutathione peroxidase (NS-GPx) (Fisher *et al*., 1999). The oxidative damage caused by ROS in cells and tissue leads to several physiological and pathophysiological effects, such as membrane lipid peroxidation, inflammation and carcinogenesis, etc. Thus, lipid hydroperoxides have been shown to inhibit DNA replication, resulting in the inhibition of cell proliferation, which finally may lead to atrophy of organs and tissues (Fukuda *et al*., 1991). Lipid peroxidation may lead to biomembrane decomposition and thus to a loss of cellular and subcellular

integrity (Kagan *et al*., 1977). When this process takes place in a controlled manner it may have beneficial effects, e.g. mitochondrial breakdown during rabbit reticulocyte maturation (Rapoport and Schewe, 1986) and differentiation of central fibre cells of the eye lens (van Leyen *et al*., 1998).

Reactive oxygen species exhibit both activating and inhibitory effects on the eicosanoid metabolism. In blood vessel microsomes the enzyme prostaglandin I_2 (PGI_2) synthetase is selectively inhibited by ROS whereas thromboxane A_2 (TXA_2) synthetase is unaffected (Schimke *et al*., 1992). Experiments with Se-deficient bovine endothelial cells revealed that the production of the vasoconstrictor and platelet-proaggregator TXA_2 was markedly increased while the production of the vasodilator and inhibitor of platelet aggregation PGI_2 was significantly decreased (Cao *et al*., 2000). Sumiya *et al*. (1993) found that the inhibition of 12-HETE, thromboxane B_2 and 12-hydroxy-5,8,10-heptadecatrienoic acid (HHTrE) from AA by xanthine plus xanthine oxidase could be reversed by the addition of catalase, thus supporting the role of the hydroperoxide tone in regulating platelet prostaglandin endoperoxide synthase and lipoxygenase activities. Oxidative stress affects AA metabolism at several levels, with PGI_2 synthesis being more sensitive than then TX and LOX pathways.

1.5 HETEROGENEITY OF 12-HpETE REDUCING ENZYMES

In platelets 12-HpETE is barely detectable as it is rapidly reduced to 12-HETE, the major product of the 12-LOX pathway, and other catabolites (Bryant *et al*., 1982). The selenium-dependent glutathione peroxidases, e.g. GPx-1 and PHGPx (Fig. 6), and the selenium-independent glutathione transferases are capable of reducing 12-HpETE. It has been shown that platelets from selenium-deficient rats produce a sevenfold greater amount of 12-HpETE than platelets from control animals, thus indicating that the selenium-dependent glutathione peroxidases coupled to the hexose monophosphate shunt play an important role in the enzymatic reduction of hydroperoxides (Bryant *et al*., 1982, 1983).

1.5.1 Selenium-dependent Peroxidases

The family of selenium-dependent peroxidase enzymes consist of:- (i) GPx-1 (Mills, 1957; Flohé *et al*., 1973; Rotruck *et al*., 1973), (ii) PHGPx (Ursini *et al*., 1982), (iii) plasma glutathione peroxidase (pGPx) (Maddipati and Marnett, 1987, Takahashi *et al*., 1987), and (iv) gastrointestinal glutathione peroxidase (GI-GPx) (Chu *et al*., 1993). These enzymes contain selenocysteine at their active site. All glutathione peroxidases reduce H_2O_2 and alkyl

hydroperoxides but they differ in their specificities for other hydroperoxides. Thus PHGPx reacts with phospholipid hydroperoxides, whereas GPx-1 is inactive towards this substrate. The activity of GPx-1 is higher in most cells and tissues than that of PHGPx (Zhang *et al*., 1989).

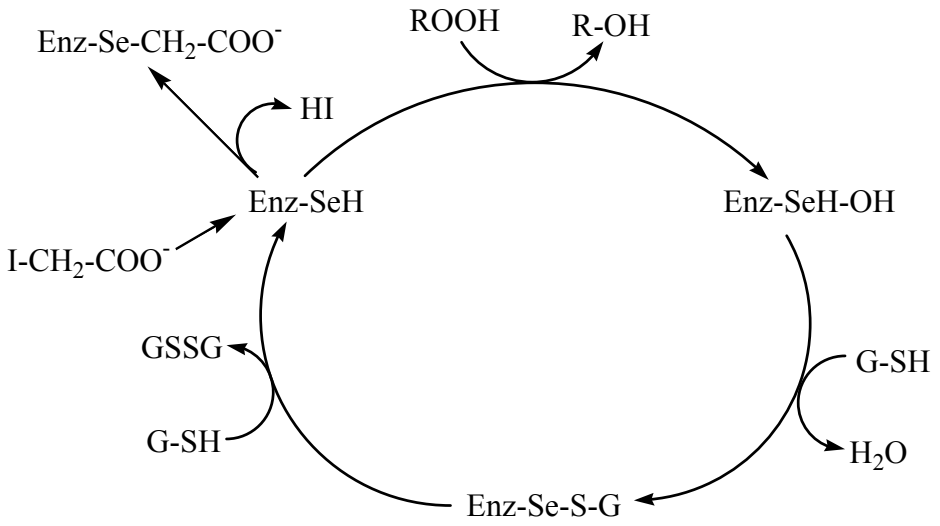

Figure 6. Glutathione peroxidase-catalysed reduction of hydroperoxy-polyenoic fatty acids and its inhibition by the carboxymethylating agent iodoacetate.

Another selenoprotein, thioredoxin reductase (TR), which possesses homology to glutathione reductase at the DNA level, has been shown to directly reduce lipid hydroperoxides (Bjornstedt *et al*., 1997). This enzyme also reduces selenite to selenide, which inhibits lipoxygenases.

1.5.1.1 LOCALISATION AND STRUCTURE

The GPx-1 and PHGPx are found in brain, liver, heart and other tissues. GI-GPx is found exclusively in the gastro-intestinal tract (Chu *et al*., 1993) and pGPx is found in various tissues in contact with body fluids e.g. kidney and maternal/fetal interfaces (Yoshimura *et al*., 1991a, b; Avissar *et al*., 1994).

With the exception of PHGPx, which is a monomeric protein, the other glutathione peroxidases occur as tetramers. PHGPx is synthesised as a long form (L-form, 23 kDa) which

contains a leader sequence for transport to the mitochondria and a short form (S-form, 20 kDa) which occurs in the cytoplasm (Arai *et al*., 1999).

1.5.1.2 ROLE OF SELENIUM STATUS

GPx-1 and PHGPx are dependent on selenium for their activity. The oxidative challenge to selenium deficient cells or tissues result in more damage to cells and tissues as compared to the controls. In selenium-deficient rat basophilic leukemia cells the syntheses of GPx-1 and PHGPx require different levels of selenium (Weitzel and Wendel, 1993). After 5 days of incubation under Se-deficiency, GPx-1 showed a total loss of its activity whereas the PHGPx retained 30% activity. Upon supplementation by selenium, PHGPx activity returned to 100% of the control after 8 h while the GPx-1 activity was restored to 100% after 164 h. Weitzel *et al*. (1990) and Brigelius-Flohé *et al*. (1997) reported that different mouse organs and cell lines respond individually to selenium depletion and supplementation. The human T cell clone Jurkat did not respond at all with an increase of GPx-1 or PHGPx activity upon selenium supplementation. In the murine T cell clone EL4 6.1 the GPx-1 selectively increased upon selenium supplementation whereas PHGPx activity remained marginal even after 3 days.

1.5.1.3 ROLE OF GPx-1

The cellular glutathione peroxidase, GPx-1, is the major isoform and is ubiquitously distributed. GPx-1 is more important in the defence against initiation of lipid peroxidation in a concerted action with phospholipases suggesting a complementary role in hydroperoxide reduction (Ursini *et al*., 1985; Ursini and Bindoli, 1987; Thomas *et al*., 1990). Most studies on the role of glutathione peroxidases have been carried out using animals fed with a selenium-deficient diet, but the selenium deficiency also affected the activity of several other proteins including PHGPx. *In vitro* studies have shown that enhanced GPx-1 activity inhibits hydroperoxide-induced apoptosis (Kayanoki *et al*., 1996; Packham *et al*., 1996). In experiments with GPx-1 knock-out mice, neither increased sensitivity to hyperoxia, nor retarded rates of H_2O_2 reduction and increased lipid peroxidation were observed as compared with the wild-type mice (Ho *et al*., 1997). The platelets from the GPx-1 knock-out mice, however, exhibited an altered AA metabolism, generated less 12-HETE and more polar products, such as epoxy alcohol and trihydroxy derivatives. GPx-1 is functionally associated with the 12-LOX pathway and efficiently converts 12-HpETE to 12-HETE (Bryant and Bailey, 1980; Bryant et al., 1982).

1.5.1.4 ROLE OF PHGPx

The exact role of PHGPx is not fully understood (Brigelius-Flohé *et al*., 1997). Initial observations suggested a specific role of PHGPx in the intracellular differentiation, and as an antioxidant. The levels of PHGPx have been found to increase during sperm transition from round to elongated form (Maiorino *et al*., 1998). It exists as a soluble peroxidase in spermatids but persists as an enzymatically inactive, oxidatively cross-linked, insoluble protein during further sperm maturation (Ursini *et al*., 1999). Imai *et al*. (1996) showed that the over-expression of PHGPx in rat basophile leukemia cells suppressed the cell death due to oxidative damage. The over-expression of PHGPx in endothelial cells cultured in selenium-rich medium however, did not contribute significantly to the H_2O_2-induced cytotoxicity. But, in the absence of selenium a marked reduction in the cytotoxicity was observed (Brigelius-Flohé *et al*., 1997). In contrast, the selenium deficiency in liver did not result in any apparent oxidative damage (Ursini *et al*., 1997).

1.5.2 The Selenium-independent Enzymes

The selenium-independent enzymes include the complex family of glutathione transferases (GSTs), including mouse lung, human and rat liver microsomal, human Alpha, Mu and Theta classes. The GSTs exhibit glutathione peroxidase activity towards phospholipid hydro-peroxides (Hurst *et al*., 1998). These enzymes exhibit a lower specific activity towards phospholipid hydroperoxides than PHGPx.

Recently, a non-selenium glutathione peroxidase (NS-GPx, also referred to as 1-Cys peroxiredoxin), which belongs to the thioredoxin peroxidase family has been identified (Fisher *et al*., 1999). It is capable of reducing both H_2O_2 and organic hydroperoxides, including phospholipid hydroperoxides. This protein has been shown to be identical to the acidic Ca^{2+}-independent PLA_2 found in human and bovine lung (Chen *et al*., 2000b), thus exhibiting bifunctional catalytic properties, i.e. the regulation of phospholipid turnover as well as the protection against oxidative injury.

1.6 REGULATION OF THE 12-LOX PATHWAY BY SELENO-ENZYMES

Plenty of evidence has accumulated which illustrated that the glutathione peroxidases are intimately integrated in the regulation of arachidonic acid metabolism in mammalian cells. The selenium-dependent phospholipid hydroperoxide glutathione peroxidase (PHGPx), which is found in almost every cell, has been shown to play an important role in the 5-LOX pathway

(Weitzel and Wendel, 1993). Selenium-deficient rat basophilic leukemia cells produced 8-fold higher amounts of the lipoxygenase products, including 5-HpETE, which was otherwise undetectable in control cells. These findings suggested that the PHGPx regulated the 5-LOX activity by modulating the hydroperoxide tone for the production of leukotrienes (Weitzel and Wendel, 1993; Imai *et al*., 1998). A similar role for PHGPx was demonstrated for the regulation of 15-LOX (Schnurr *et al*., 1996) and cyclooxygenase (Sakamoto *et al*., 2000).

Several reports (Calzada *et al*., 1992; Rey *et al*., 1994; Lagarde *et al*., 1995; Lemaitre *et al*., 1997) suggest that the glutathione peroxidases may play a role in fine-tuning the physiological concentration of 12-HpETE in platelets. For the regulation of the intracellular eicosanoid metabolism GPx-1 and PHGPx may be considered. In platelets, there is ample evidence for a crucial role of glutathione peroxidases in the arachidonic acid metabolism, which was solely described to GPx-1 (Bryant and Bailey, 1980; Bryant *et al*., 1982; Lagarde *et al*., 1995). Bryant and Bailey (1980) showed that a limited glutathione peroxidase activity resulted in an increase in the LOX derived trihydroxy fatty acids and concluded this was due to the limited reduction of 12-HpETE by glutathione peroxidases.

The mammalian 5-, 12- and 15-lipoxygenases are known to translocate to intracellular membranes in activated cells (Hagmann *et al*., 1996; Pouliot *et al*., 1996; Brinckmann *et al*., 1998). The primary metabolites, the hydroperoxy fatty acids, are thus formed at a membrane compartment. PHGPx possesses an affinity to bind to membranes (Chambers *et al*., 1994). Thus PHGPx has been shown to play a greater role than GPx-1 in regulating the 5-LOX pathway in leukocytes (Weitzel and Wendel, 1993). The role of PHGPx in the regulation of the 12-LOX pathway remains unclear.

1.6.1 Effect of Selenium Deficiency on the 12-LOX Pathway

Bryant and Bailey (1980) observed decreased levels of 12-HETE formation in selenium-deficient rat platelets. In contrast, Maddox *et al*. (1991) found increased levels of 12-HETE in calcium ionophore-stimulated selenium-deficient bovine lymphocytes. Depletion of the GPx activity by limiting the reduced glutathione has been shown to cause enhanced arachidonic acid oxygenation (Calzada *et al*., 1991). These divergent results have been explained in the following way:- (a) lowering the hydroperoxide tone inhibits the 12-LOX activity while favouring the reduction of 12-HpETE to 12-HETE, and (b) increasing the hydroperoxide tone stimulates 12-LOX activity while diverting 12-HpETE to the hepoxilins

(see fig. 5). Since the increased hydroperoxide tone facilitates the inactivation of 12-LOX the sum of both effects result in a decrease in 12-HETE formation.

The level of GPx-1 plus PHGPx and 12-HpETE in the cell determines the amount of 12-HETE and hepoxilins produced. Under conditions of high 12-HpETE levels it can be hypothesised that a high level of selenium-containing glutathione peroxidases would result in the reduction of most of the 12-HpETE to 12-HETE, and conversely, a low level of selenium-containing glutathione peroxidases would increase substrate availability for the isomerase pathway resulting in increased hepoxilin synthesis. The latter condition may be induced in cells by (i) carboxymethylation of selenium-containing glutathione peroxidases by iodoacetate (Fig. 6) (Ursini *et al*., 1985), (ii) a reduced glutathione supply by the addition of glucose (2 mg/ml) (Bryant and Bailey, 1979), (iii) by culturing cells in selenium-deficient medium (Weitzel and Wendel, 1993) or (iv) glutathione depletion by the addition of diamide or diethyl maleate (Bryant *et al*., 1982). In this manner, the pathway may be shifted towards the isomerisation route.

1.6.2 PHGPx as an Inhibitor of Lipoxygenases

PHGPx has been identified as the endogenous inhibitor masking 12-LOX activity in the epidermoid cell line A431 (Chen *et al*., 1997; Huang *et al*., 1998). *In vitro* studies using purified PHGPx from A431 cells revealed that platelet-type 12-LOX, reticulocyte 15-LOX and COX-2 are more sensitive to inhibition than rat 5-LOX, leukocyte-type 12-LOX and COX-1 (Huang *et al*., 1999a). Similarly, 5-LOX activity in B-lymphocytes and immature HL-60 cells has been shown to be inhibited by GPx-1 (Straif *et al*., 2000) and PHGPx (Werz and Steinhilber, 1996). The various functions ascribed to PHGPx thus suggest that it is not only a general antioxidant, rather it plays a specific role in metabolic regulation in cells and tissues.

1.7 HETEROGENEITY OF CATALYSTS INVOLVED IN HEPOXILIN SYNTHESIS

Studies on the mechanism of formation of the hepoxilins indicate that they are formed through the rearrangement of 12-HpETE (Bryant *et al*., 1982; Pace-Asciak, 1984a,b). Hepoxilin formation has been observed in rat aorta (Laneuville *et al*., 1991a), rat pineal gland (Reynaud *et al*., 1994a, b), brain hippocampus and skin subcutis layer (Pace-Asciak *et al*., 1993). The following routes have been described for the formation of hepoxilins:-

i) 12-HpETE is transformed by a hemin- or haemoglobin-assisted intramolecular rearrangement into two hepoxilins, 8(*S*,*R*)-hydroxy-11,12-epoxyeicosa-5*Z*,9*E*,14*Z*-trienoic acid (HXA_3) and 10(*S*,*R*)-hydroxy-11,12-epoxyeicosa-5*Z*,8*Z*,14*Z*-trienoic acid (HXB_3), thus indicating a nonenzymatic reaction (Pace-Asciak, 1984b). Surprisingly, the hepoxilins have been shown to be formed by cells and tissues in which 12-LOX is also present (Pace-Asciak, 1993). Epoxy alcohols may also be formed from hydroperoxy fatty acids by other inorganic agents, including strong acid and ferrous iron (Chang *et al*., 1996).

ii) Cytochrome *P*-450 has been shown to catalyse the rearrangement of hydroperoxy fatty acids via stereoselective synthesis to epoxy alcohols, i.e. hepoxilin-like compounds (Song *et al*., 1993a,b; Chang *et al*., 1996).

iii) Hepoxilin-like compounds have been shown to be formed by the hydroperoxidase activity of other enzymes. The 15-lipoxygenase (Garssen *et al*., 1971; Bryant *et al*., 1985) and a hydroperoxide isomerase from fungus (Gardner, 1991) have been shown to catalyse the formation of hydroxyepoxy fatty acids.

In 1993 it was shown that the isomerisation of 12-HpETE is a heat-sensitive process thus suggesting an enzymatic process (Pace-Asciak *et al*., 1993). Further studies with rat pineal gland using the *R*/*S*-isomers of 12-HpETE as substrate revealed that HXA_3 is exclusively derived from the 12*S*-isomer (Reynaud *et al*., 1994b), whereas in the hemin catalysed reaction HXA_3 is formed from both 12*R*- and 12*S*-isomers. Thus the authors suggested tentatively the enzyme ´hepoxilin synthase`. However, to-date this enzyme has not been isolated and characterised.

Hepoxilin B_3 is formed non-enzymatically (Reynaud *et al*., 1994b), since the stereochemical analysis of HXB_3 formation by hemin catalysis did not show any stereospecificity. The ´enzymatic` catalysis yielded mainly HXA_3, whereas the hemin-catalysed reaction produced HXA_3 and HXB_3.

1.8 BIOLOGICAL ROLE OF THE 12-LOX DERIVED EICOSANOIDS

1.8.1 Biological role of 12-HpETE and 12-HETE

Numerous papers have reported various biological effects for the 12-LOX derived metabolites 12-HpETE and 12-HETE. Both 12-HpETE and 12-HETE are capable of releasing intra-cellular calcium in human neutrophils. However, 12-HETE is a stronger agonist than 12-

HpETE (Yoshino *et al*., 1994; Powell *et al*., 1995; Reynaud and Pace-Asciak, 1997). The reduced biological activity of 12-HpETE, as compared to 12-HETE, has been shown to be due to its isomerisation to the biologically less active hepoxilins (Reynaud and Pace-Asciak, 1997). The reduction or oxidation of 12-HETE leading to 12-oxo-ETE and 12-HETrE, leads to a reduction in biological activity (Powell *et al*., 1995). In all foregoing studies, it remains unclear whether the effects observed by 12-HpETE are solely due to 12-HpETE or they are caused by the products 12-HETE and/or the hepoxilins.

In normal cells under physiological conditions the level of 12-HETE is low (Tang and Honn, 1999). The physiological functions of 12-HpETE and 12-HETE are not yet fully understood. Lin *et al*. (1996) found increased levels of 12-HETE in both the host and rejected tissue from corneal grafts. The 12-LOX product 12-HETE has been shown to transduce growth-related signals and regulate cell proliferation, survival and apoptosis (Tang *et al*., 1996). The products of the 12/15-LOX are responsible for the generation of endogenous ligands for PPAR-gamma (Huang *et al*., 1999b). Desplat *et al*. (1998) found that 12-HETE had no effect on human marrow mononuclear cells and marrow stromal cell cultures. Table 3 summarises some of these biological effects.

Table 3. Biological effects of 12-HpETE and 12-HETE

Tissue and species	Biological activity	Reference
Human leukocyte	Chemotaxis	Palmer *et al*., 1980
Human leukocyte	Induction of heat shock protein	Koller and Konig,1991
Prostate tumour cells	Increased invasion	Liu *et al*., 1997
Aplysia neurons	Increase in outward K^+ current	Buttner *et al*., 1989
Rat pineal gland	Stimulation of melatonin synthesis	Sakai *et al*., 1988
Lewis lung cells	Endothelial cell retraction	Honn *et al*., 1994b
Rat aortic smooth muscle cell	Stimulation of migration	Nakao *et al*., 1982
Melanoma cells	Cytoskeletal rearrangement	Timar *et al*., 1993a,b

12-HETE has also been found to play an important role in multiple steps of the cancer metastasis cascade. It up-regulates integrin, a surface matrix receptor, and enhances adhesion (Chopra *et al*., 1991; Timar *et al*., 1992). The changes in cytoskeletal proteins results in cell shape changes from round to spread, thus promoting tumour cell spread. Liu *et al*. (1996,

1997) showed that 12-HETE significantly enhances tumour cell motility in prostate adenocarcinoma cells and invasion in breast cancer cells. More recent studies in breast cancer (Natarajan and Nadler, 1998) and human prostate carcinoma (Nie *et al*., 1998, 1999) support the procarcinogenic role of 12-HETE. Furthermore, 12-HETE induces the secretion of lysosomal enzymes, including cathepsins, collagenase IV and heparanases, which facilitates tumour cells to cross the tissue matrix (Ulbricht *et al*., 1996; Honn *et al*., 1994c; Liu *et al*., 1996). Ding *et al*. (1999) found that inhibition of the 5- and 12-LOX in human pancreatic cancer cells, in which the levels of both enzymes are upregulated and their products stimulate mitogenesis, resulted in apoptosis.

Li *et al*. (1997) found that a decrease in glutathione levels in cortical neurons triggers the activation of 12-LOX and ultimately leads to cell death. Inhibitors of the arachidonic acid cascade and 12-LOX blocked cell death, suggesting that LOX products play a critical role in neuronal degeneration. Nigam *et al*. (1999) found lower levels of 12-HETE formation in the tissue of female patients with invasive cervix carcinoma as compared to uterine tissue from healthy women. They concluded that the hydroperoxide-reducing capacity of the invasive cervix carcinoma tissue might be enhanced which in turn might suppress the 12-LOX activity. Thus an anticarcinogenic role of 12-LOX in human cervix was proposed.

1.8.2 Biological Role of HXA_3

Lipoxygenase products of arachidonic acid have been implicated in the augmentation of glucose-dependent secretion of insulin by pancreatic cells *in vitro*. The first reported biological action of the hepoxilins was in 1984 when it was shown that HXA_3 dose-dependently stimulated glucose-induced insulin secretion from rat pancreatic islets, suggesting that HXA_3 could be the active intermediate involved in the potentiation of glucose-dependent insulin secretion by both arachidonic acid and 12-HpETE (Pace-Asciak and Martin, 1984). The trivial name hepoxilin (**H**ydroxy**EPOX**ide + **I**nsu**LIN**) has been introduced by these authors. Since then a variety of other biological effects of hepoxilins have been observed (Table 4).

Table 4. Biological effects of the hepoxilins

Tissue and species	Biological activity	Reference
Human neutrophils	Changes in intracelluar pH and membrane potential	Dho *et al*., 1990
Human neutrophils	Second messengers (AA, DAG)	Nigam *et al*., 1990b
Human neutrophils	Activation of phospholipase D	Nigam *et al*., 1993
Human neutrophils	Modulation of agonist-induced (fMLP, PAF and LTB_4) effects	Laneuville *et al*., 1993
Human neutrophils	Intracellular calcium release	Reynaud *et al*., 1999
Human leukocytes	Induction of heat shock protein	Lin *et al*., 1991
Human platelets	Regulatory volume decrease	Margalit *et al*., 1993
Human platelets	Inhibits aggregation	Margalit *et al*., 1994
Rat pancreatic cells	Insulin secretion	Pace-Asciak and Martin, 1984
Rat pineal gland	Melatonin secretion and Stimulation of adenyl cyclase	Reynaud *et al*., 1994a
Rat skin	Vascular permeability	Laneuville *et al*., 1991b
Rat aorta and vein	Modulation of vascular tone and contractility	Laneuville *et al*., 1992b
Guinea-pig trachea	Vascular contraction	Laneuville *et al*., 1992a
Guinea pig visceral yolk sac	Calcium transport	Derewlany *et al*., 1984
Hippocampal CA1 neurons	Neuromodulation	Carlen *et al*., 1989, 1994
Aplysia neurons	Second messengers	Piomelli *et al*., 1987
Aplysia neurons	Second messengers	Piomelli, 1991
Aplysia neurons	Membrane hyperpolarization	Volterra *et al*., 1992

The main action of HXA_3 is believed to be mediated by its ability to release intracellular calcium (Dho *et al*., 1990). Hepoxilin A_3 causes a dose-dependent rapid, transient elevation of Ca^{2+} from intracellular stores in human neutrophils. The HXA_3 induced calcium release is less pronounced than by both 12-HETE and 12-HpETE. This effect is blocked by pertussis toxin, suggesting the involvement of receptors coupled to GTP-binding proteins. Treatment of [1-^{14}C]-arachidonic acid labelled neutrophils with HXA_3 resulted in a time- and concentration-dependent release of the second messengers AA and diacylglycerol (Nigam *et*

al., 1990b). The release of the second messengers could also be blocked by pertussis toxin thus indicating a receptor-mediated event. Competitive binding experiments demonstrated that human neutrophils contain specific binding sites for HXA_3 (Reynaud *et al*., 1995a, 1996). The specific binding could be inhibited in broken membranes by proteinase K pretreatment, however, binding was unaffected in intact cells. These results suggest that the actions of hepoxilin are mediated via a still unidentified intracellular hepoxilin-binding protein (Reynaud *et al*., 1995b). The HXA_3 potentiated fMLP-induced release of AA occurs via activation of the phospholipase D pathway (Nigam *et al*., 1990b).

A short-lived lipoxygenase-derived product has been shown to control regulatory volume decrease (RVD). Buttner *et al*. (1989) and Volterra and Siegelbaum (1990) showed that 12-LOX metabolites directly open S-type K^+ channels of *Aplysia* sensory neurons. They concluded that it was a downstream metabolite of 12-HpETE other than 12-HETE. This product was identified as hepoxilin A_3 and regulates K^+ and Cl^- channels. In platelets, HXA_3 antagonises the cell volume expansion (Margalit and Livne, 1992; Margalit *et al*., 1993). This phenomenon known as ´regulatory volume decrease` by HXA_3 is regulated by K^+ channels.

The HXA_3 epimers have been shown to possess different biological effects. Thus, the *R* epimer only potentiated bradykinin induced vascular permeability, whereas the *S* epimer potentiated norepinephrine-induced vascular contraction in the aorta and portal vein (Laneuville *et al*., 1992b). However, both epimers are capable of antagonising volume expansion in platelets (Margalit *et al*., 1993).

In earlier reports and reviews it has been suggested that unlike its methyl ester, the free acid form of HXA_3 is unable to enter neutrophils and other cells. The majority of biological effects reported with regards to HXA_3, therefore, have been demonstrated with the methyl ester. Recently, Reynaud *et al*. (1999) reported that the free acid causes a rise in intracellular calcium in human neutrophils and that the previously observed impermeability of HXA_3 was due to DMSO, which was used as a solvent. DMSO formed a polar hydrogen bond with HXA_3.

1.9 ROLE OF HEPOXILIN IN DISEASE

Earlier studies revealed that HXA_3 caused a stimulation of the glucose-evoked release of insulin from perfused pancreatic islets of Langerhans *in vitro* (Pace-Asciak and Martin, 1984).

Subsequently, it was shown that the cells are capable of producing HXA_3 from endogenous sources as well as 12-HpETE indicating that the compound is a potential endogenous regulator of insulin secretion (Pace-Asciak *et al*., 1985, 1986). Bolus intravenous injection of arachidonic acid in rats led to the appearance of HXA_3 in blood (Pace-Asciak *et al*., 1987). Simultaneously, the plasma concentration of insulin increased by 36% thus demonstrating the formation of this insulin secretagogue *in vivo* and its correlation with plasma insulin. Further experiments in a genetic rat model of type I insulin-dependent diabetes revealed higher HXA_3 and thromboxane B_2 concentrations in the blood as compared to control animals (Pace-Asciak et al., 1988). To determine whether HXA_3 is active *in vivo*, Pace-Asciak and co-workers administered the hepoxilins intra-artetially in anaesthetized rats (Pace-Asciak *et al*., 1999). The hepoxilin resulted in a rapid rise in blood insulin levels, however, the release was found to be dependent on the glucose status. Hepoxilin was found to be 50× more effective in releasing insulin as compared to glucose. Taken together, these results suggest that hepoxilins may represent new compounds as therapeutics in type II diabetes mellitus.

In numerous diseases oxidative stress has been shown to play a critical role. Oxidative stress is due to a shift in the peroxide tone, that is, the steady-state between hydroperoxide generating systems and the hydroperoxide scavenging systems, e.g. GPx-1 and PHGPx (see section 1.6.1). Ultimately, this may lead to a shift in the 12-LOX pathway towards the isomerisation route yielding the hepoxilins.

Most of the studies investigating the effects of free radical injury on the vascular system have been performed in animal models. In Se-deficient rats, it has been observed that an inverse correlation exists between glutathione peroxidase activity and lipid peroxide levels, as the peroxidase activity decreased the lipid peroxide levels increased (Qu *et al*., 2000). Furthermore significant increases in the levels of serum total cholesterol and low-density lipoprotein cholesterol were observed, supporting the hypothesis that Se-status (and thus glutathione peroxidase) and lipid peroxidation are involved in the etiology of cardiovascular disease. In a further study using Se-deficient rats, which exhibited 87% reduction in mitochondrial and cytosolic glutathione peroxidase activities, increased peroxidation in heart homogenates was observed (Molina and Garcia, 1997). No peroxidation occurred through inhibiting cytsolic catalase, suggesting that the selenoenzyme glutathione peroxidase appears to be more important for detoxification of H_2O_2 in the heart. However, in the liver of Se-deficient rats, PHGPx has been shown to be much more crucial than GPx-1 in preventing the

elevation of lipid peroxides (Guan *et al*., 1995). A study in 58 humans with congestive heart failure also exhibited significantly increased levels of plasma lipid peroxides and decreased levels of glutathione peroxidase (Keith *et al*., 1998).

It is well known that brain and nervous system cells are prone to oxidative damage because of their relatively low antioxidant content and high levels of membrane polyunsaturated fatty acids (Syburra and Passi, 1999). The erythrocyte antioxidant enzymes, Se-dependent glutathione peroxidase, glutathione reductase and superoxide dismutase, all exhibit significant reduction in activity in various brain tumours (Rao *et al*., 2000) suggesting that the antioxidant enzymes have a role in the onset of oxidative stress in brain tumours. The erythrocytes of patients with multiple sclerosis exhibit significantly reduced levels (approximately 60% as compared to controls) of glutathione peroxidase whereas the levels of arachidonic acid are significantly higher (Syburra and Passi, 1999). Similarily, the levels of plasma-GPx have been found to be reduced by approximately 60% in haemodialysis patients and thus may contribute to atherogenesis in renal failure (Roxborough *et al*., 2000).

In view of the reported biological effects of hepoxilin in various cells/tissues and insulin secretory activity in diabetic rats, the role of hepoxilin in diseases where oxidative stress plays an imporant role remains to be clarified.

1.10 OBJECTIVES

The biochemical mechanism of isomerisation of 12-HpETE to hepoxilin as well as the regulation of this process is not understood clearly. In experiments with rat pineal glands (Pace-Asciak *et al*., 1993; Reynaud *et al*., 1994a,b), the authors suggested the involvement of an enzyme, so-called 'Hepoxilin synthase'. This enzyme, however, has not been isolated and characterised so far. Whereas soybean lipoxygenase-1 and reticulocyte 15-lipoxygenase have been shown to catalyse the formation of hydroxyepoxy fatty acids (Garssen *et al*., 1971; Bryant *et al*., 1985), the role of mammalian 12-LOX in the isomerisation of 12-HpETE has not yet been investigated. Nevertheless 12-LOX, which possesses not only the dioxygenase activity but also hydroperoxidase activity, it is reasonable to assume that the synthesis of hepoxilins may also be catalysed by the latter activity. An analogues reaction is observed in the synthesis of lipoxins (Ueda *et al*., 1987, Wiseman *et al*., 1987) and LTA_4 (Rouzer *et al*., 1986). The present thesis deals with this phenomenon using pure enzyme.

With respect to the biosynthesis and regulation of hepoxilin formation, two factors play a critical role:- first, the concentration of 12-HpETE, which is the common precursor for both 12-HETE and hepoxilin formation, and second the hydroperoxide tone which exerts regulatory effects on the 12-LOX pathway. It can therefore be assumed that the hepoxilin synthesis is regulated by changes in the PHGPx activity. In A431 cells, it has been shown that the 12-LOX activity is masked by an endogenous inhibitor identified as PHGPx (Chen *et al*., 1997; Huang *et al*., 1998). It is therefore tempting to speculate that PHGPx and the hydroperoxide tone may be implicated in the regulation of the 12-LOX pathway. In the present study this aspect is investigated extensively.

Whereas the biological role of 12-HETE in mammalian cells has been well characterised (Koller and Konig, 1991; Honn *et al*., 1994a,b; Natarajan and Nadler, 1998; Reynaud and Pace-Asciak, 1997; Wen *et al*., 2000), the role of HXA_3 has not been studied to the same extent. Numerous publications and review articles exist on the biological effects of HXA_3 on signal transduction in human neutrophils (Dho *et al*., 1990; Nigam *et al*., 1990b; Pace-Asciak, 1994; Pace-Asciak and Nigam, 1991; Pace-Asciak *et al*., 1999). Inasmuch as the majority of studies were performed with the methyl-ester of HXA_3 and not with the free acid, the biological effects of HXA_3 free acid in intact human neutrophils have been presented.

In summary, the purpose of this study was:-

I. to show the synthesis of HXA_3 in various cells, including human platelets, the epidermoid carcinoma cell line A431 and the acute myeloid leukemia cell line UT-7;
II. to investigate the possible interaction between the mammalian type 12-lipoxygenases and the glutathione peroxidases GPx-1 and PHGPx for the biochemical synthesis of HXA_3 formation using arachidonic acid and 12-HpETE as substrate;
III. to study the biological effects of HXA_3 on signal transduction processes in human neutrophils; and
IV. to study the biological effects of HXA_3 on tumour cells.