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Review

Hepoxilin A_3 synthase

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Abstract

Hepoxilins constitute a group of 12*S*-hydroperoxyeicosatetraenoic acid (12S-HpETE)-derived epoxy-hydroxy fatty acids that have been detected in various cell types and tissues. Although hepoxilin A_3 (HXA_3) exhibits a myriad of biological activities, its biosynthetic mechanism was not investigated in detail. Here we review the isolation, cloning, and characterization of a leukocyte-type 12*S*-lipoxygenase (12S-LOX) from rat insulinoma cells RINm5F, which exhibits an intrinsic hepoxilin A_3 synthase activity. Confirmation for this observation was achieved by coimmunoprecipitation of HXA_3 synthase activity with an anti-leukocyte 12S-LOX antibody, preparation of recombinant rat 12S-LOX enzyme from RINm5F cells, and assay of HXA_3 synthase activity therein. Site-directed mutagenesis studies performed on rat 12S-LOX showed that 12-lipoxygenating enzyme species exhibit a strong HXA_3 synthase activity that is impaired when the positional specificity of arachidonic acid is altered in favor of 15-lipoxygenation. Inasmuch as cellular glutathione peroxidases (cGPx and PHGPx) and HXA_3 synthase compete for the same substrate 12S-HpETE, it can be proposed that the overall activity of glutathione peroxidases, representing the overall peroxide tone, finely tunes the rate of HXA_3 formation.

Keywords: Hepoxilin synthase; 12*S*-Lipoxygenase; Glutathione peroxidase; Hepoxilin A3; Rat insulinoma cells; Site-directed mutagenesis

Introduction

Biosynthesis

Hepoxilins are epoxy-hydroxy eicosanoids synthesized through the 12*S*-lipoxygenase (12S-LOX) pathway of the arachidonic acid (AA) metabolism. AA after dioxygenation to 12*S*-hydroperoxy-eicosatetraenoic acid (12S-HpETE) is bifurcated in a reduction route leading to 12S-HETE formation and an enzymatic route, the hepoxilin synthase pathway, giving rise to formation of hepoxilins (Fig. 1). Major hepoxilins formed are the bioactive hepoxilin A_3 (HXA_3) (8*S*/*R*-hydroxy-11,12-epoxyeicosa-5*Z*,9*E*,14*Z*-trienoic acid) and the inactive HXB_3 (10*S*/*R*-hydroxy-11,12-epoxyeicosa-5*Z*,8*Z*,14*Z*-trienoic acid) [1,2]. Hepoxilin-like compounds have also been obtained by the cytochrome P-450-mediated catalysis of the stereospecific rearrangement of hydroperoxy fatty acids [3–5]. Moreover, enzymes, such as 15-LOX and fungal hydroperoxide isomerase, which exhibit the hydroperoxidase activity, have been shown to catalyze the biosynthesis of hepoxilins [6–8].

Formation of hepoxilins has been demonstrated in platelets [9–11], lung [12,13], pancreatic islets [14,15], brain [16], aorta [17], neutrophils [18], *Aplysia* brain [19], and rat insulinoma cells [20]. Hepoxilin-like compounds (14,15-hepoxilins) derived from 15S-HpETE and from docosahexaenoic acid (bis-α-dihomo-hepoxilins) have also been isolated from garlic roots [21], and pineal glands and brain, respectively [22].

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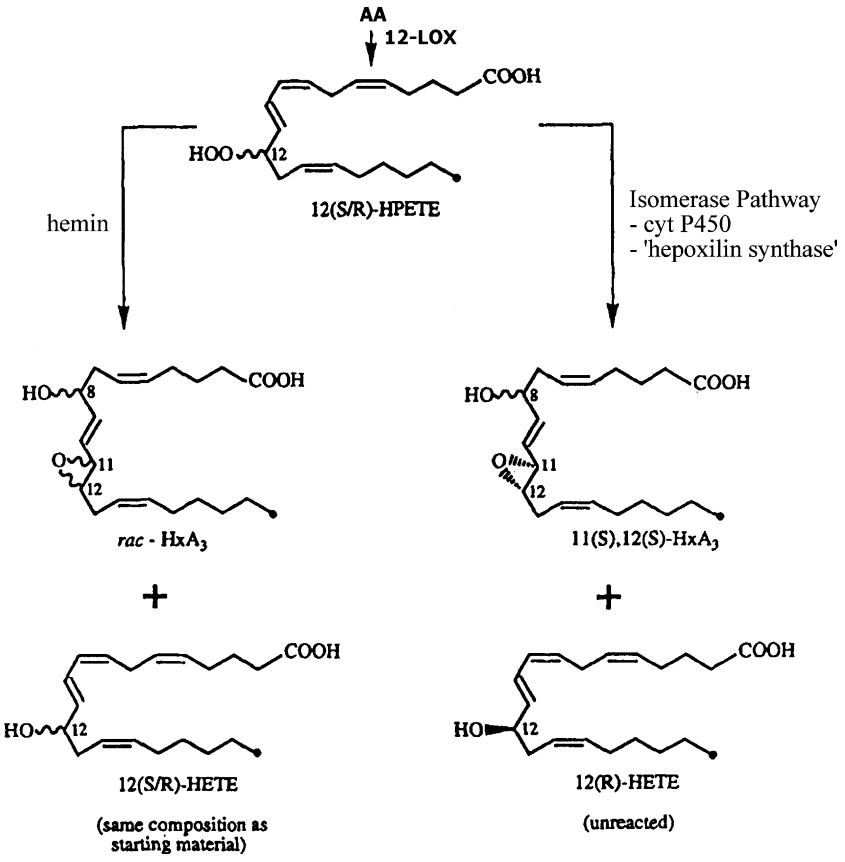

Fig. 1. Scheme describing the biosynthesis of hepoxilins.

Metabolism

The catabolism of hepoxilins may occur via three different pathways:

(a) via inherent epoxide hydrolase activity to biologically inactive trihydroxy products 8,11,12-trihydroxy-5*Z*,9*E*,14*Z*-trienoic acid (8,11,12-TriHETrE) and 8,11,12-trihydroxy-5*Z*,10*E*,14*Z*-trienoic acid (8,9,12-TriHETrE), trivially named trioxilins [9,23,24],
(b) via addition of glutathione by liver glutathione-*S*-transferase into a bioactive peptido-hepoxilin A_3 conjugate (HXA_3-C) [25],
(c) via apparently a specific ω-hydroxylase found in human neutrophils resulting in the corresponding ω-hydroxy metabolites [22,26]. Seemingly, this ω-hydroxylase activity differs from that required for the ω-oxidation of leukotriene B_4 [22].

Biological role of hepoxilins

HXA_3 is a potent lipid mediator, which stimulates the glucose-induced insulin secretion dose-dependently in rat pancreatic islets [14], hyperpolarizes the membrane potential as well as the postsynaptic potential in *Aplysia* and mammalian neurons [21,28,29], and regulates the cell volume in platelets and *Aplysia* neurons by activating K^+ channels [30,31]. In neutrophils, HXA_3 stimulates the intracellular calcium release from calciosomes [18,32], the heat shock protein 72 (HSP72) [33], as well as AA release and diacylglycerol formation [34,35]. Latter processes occur seemingly through G-protein-coupled receptors [34]. Recently, a stable analogue of HXA_3 has been shown to cause apoptosis in K562 cells [36]. A myriad of biological activities exhibited by HXA_3 are summarized in Table 1.

Molecular biology of hepoxilin A_3 synthase

Evidence for the presence of HXA_3 synthase activity in RINm5F cells

The biosynthesis of hepoxilins by the isomerization pathway was always highly controversial. However, in the last decade several reports were published which pinpointed the HXA_3 synthesis to be enzyme-controlled for two reasons: (1) isomerization of 12S-HpETE to hepoxilins was heat-sensitive [37], and (2) rat pineal glands converted exclusively 12S-HpETE, but not 12R-HpETE, to bioactive HXA_3 [38]. But, the involved "isomerase" was not isolated and characterized by these authors. Moreover, it was a matter of discussion whether the two enzymes 12S-LOX and "hepoxilin synthase" are coupled or are located in different compartments in the cell. Interestingly, no hepoxilin synthesis could be detected in a

Table 1
Biological activities of hepoxilins

Cell type and tissue	Biological effect	Reference
Human neutrophils	Changes in intracellular pH and membrane potential	Dho et al. [18]
Human neutrophils	Second messengers (AA, DAG)	Nigam et al. [34]
Human neutrophils	Activation of phospholipase D	Nigam et al. [68]
Human neutrophils	Modulation of agonist-induced (fMLP, PAF, and LTB_4) effects	Laneuville et al. [69]
Human neutrophils	Intracellular calcium release	Reynaud et al. [21] Sutherland et al. [32]
Human leukocytes	Induction of heat shock protein	Lin et al. [33]
Human platelets	Regulatory volume decrease	Margalit et al. [30]
Human platelets	Inhibition of aggregation	Margalit et al. [70]
Rat pancreatic cells	Insulin secretion	Pace-Asciak and Martin [14]
Rat pineal gland	Melatonin secretion and adenylate cyclase stimulation	Reynaud et al. [71]
Rat skin	Vascular permeability	Laneuville et al. [17]
Rat aorta and vein	Modulation of vascular tone and contractility	Laneuville et al. [72]
Guinea pig trachea	Vascular contraction	Laneuville et al. [72]
Guinea pig visceral yolk Sac	Calcium transport	Derewlany et al. [73]
Hippocampal CA1 neurons	Neuromodulation	Carlén et al. [29,74]
Aplysia neurons	Second messengers	Piomelli et al. [75]
Aplysia neurons	Second messengers	Piomelli [27]
Aplysia neurons	Membrane hyperpolarization	Volterra et al. [76]
K562 leukemia cells	Apoptosis by hepoxilin analogue	Qiao et al. [36]
RINm5F insulinoma cells	Pro- and anti-apoptotic reaction	Zafiriou et al. [67]

mammalian cell or tissue system, if the 12S-LOX activity was absent [20,38].

Lipoxygenases are bifunctional enzymes and exhibit both fatty acid dioxygenase and lipohydroperoxidase activities. The latter activity unlike the dioxygenase activity does not insert oxygen into the fatty acid. Thus, enzymatic transformation of AA with soybean LOX-1 [6] and reticulocyte-type 15-LOX-1 [7] also leads to hydroxy–epoxy derivatives from the preformed hydroperoxy polyenoic fatty acids. Although the platelet-type 12S-LOX does not possess any lipohydroperoxidase activity, lysates from selenium-deficient platelets were found to produce hydroxy-epoxy derivatives of AA, currently known as trioxilin A_3 and trioxilin B_3 [39]. Few years ago our laboratory reported for the first time that the conversion of AA to hepoxilin A_3 and hepoxilin B_3 is heme- and not enzyme-catalyzed. More importantly, we could demonstrate that the cytosolic glutathione peroxidase (cGPx) and the membrane-bound phospholipid hydroperoxide glutathione peroxidase (PHGPx), which are present in abundance in platelets, prevent the detection of hepoxilins by shifting the 12S-HpETE → hepoxilin pathway to 12S-HpETE → 12S-HETE pathway [40]. These findings revealed the pivotal role of selenoenzymes in the biosynthesis of hepoxilins as well as in the regulatory network of AA metabolism as a whole (Fig. 2).

Rat insulinoma cells RINm5F, which possess leukocyte-type 12S-LOX and are devoid of cGPx and PHGPx [41], are therefore capable of producing large amounts of HXA_3 when incubated with AA or 12S-HpETE [20] (Fig. 3). The HXA_3 formation was however completely abrogated when RINm5F cells stably transfected with cGPx or PHGPx were incubated with the above substrates. Strikingly, no HXA_3 was detected in the heat-inactivated lysate of RINm5F cells upon incubation with 12S-HpETE

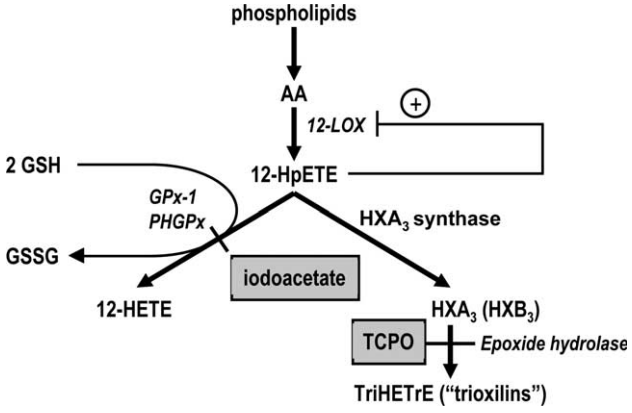

Fig. 2. Bifurcation of 12S-HpETE metabolism via reduction and isomerization routes.

or AA [20], suggesting the presence of a HXA_3 synthase-like activity regulated by glutathione peroxidases. Surprisingly, heat-denatured RINm5F cells also did not exhibit any 12S-LOX activity, which confirmed our assumption that the rat 12S-LOX exhibits an intrinsic HXA_3 synthase-like activity, which is finely tuned by cellular glutathione peroxidases. Moreover, immunoprecipitation of the 12S-LOX enzyme from RINmF5 cell lysate by the specific 12S-LOX antibody exhibited two catalytic activities, e.g., 12S-LOX and HXA_3 synthase activities in the immunoprecipitate, whereas the RINm5F cell lysate- and immunoprecipitate supernatants were deprived of both activities, suggesting that the in vivo formation of HXA_3 involves two consecutive LOX-catalyzed steps, i.e., formation of 12S-HpETE by 12S-LOX followed by isomerization mediated by HXA_3 synthase [42]. This was also supported by the colocalization of both activities in cytosol as determined by the assay of both 12S-LOX and HXA_3 activities in various subcellular fractions obtained by differential

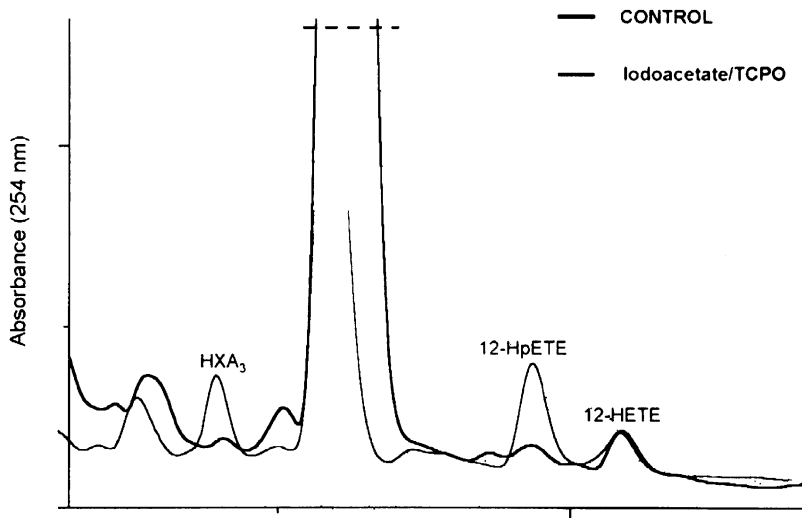

Fig. 3. HPLC profiles of hepoxilin A_3 synthesis from rat insulinoma cells (RINm5F) in the absence and presence of inhibitors of glutathione peroxidases (iodoacetate) and epoxide hydrolase (trichloropropylene oxide) [40]. The large peak between HXA_3 and 12S-HpETE peaks describes the unmetabolized AA.

centrifugation of the cell lysate [42]. The dominant role of glutathione peroxidases was excellently demonstrated by the absence of HXA_3 and 12S-HETE formation from AA or 12S-HpETE in HeLa cells overexpressing the rat 12S-LOX activity, because cGPx and PHGPx, which are abundantly present in HeLa cells, completely down-regulated the 12S-LOX activity. On the other hand, stimulation of 12S-LOX and HXA_3 synthase activities could be observed when overall hydroperoxide tone in the cell was increased by depletion of cGPx and PHGPx by diethyl maleate (DEM) in HeLa cells [20]. Similar regulations by glutathione peroxidases have also been reported for other LOXs in the literature [39,43,44].

Recently, a mammalian epidermal LOX-type 3 (eLOX3) has been reported to provide a functional link with 12R-LOX. Thus, eLOX3 transforms the 12R-LOX-derived product 12R-HpETE into a specific epoxy alcohol product, 8*R*-hydroxy-11*R*,12*R*-epoxyeicosa-5*Z*,9*E*,14*Z*-trienoic acid, which is nothing else but the *R*-isomer of HXA_3 [45]. Although eLOX3 is clearly a member of the LOX gene family, the complete absence of typical oxygenase activity under any of the variety of conditions makes it an outsider among LOXs.

cDNA cloning and characterization of HXA_3 synthase from RINm5F cells [42]

Rat 12S-LOX cDNA was cloned from mRNA prepared from RINm5F cells by RT-PCR using following primers: 5′-**cgacatatgtgtctaccgcatccgc**-3′ (forward primer) and 5′-**tggctcgagtcagatggccacgctgtt**-3′ (reverse primer). The purified 12S-LOX PCR fragment was cloned into the *Nde*I and *Xho*I sites in PET 15b bacterial expression vector. The sequence insert was confirmed by automated fluorescence sequencing. Comparison of the entire sequence with the published rat 12/15S-LOX sequence (Accession No. NM_031010) using BLAST server revealed the authenticity of the rat 12S-LOX possessing an intrinsic HXA_3 synthase activity. In order to obtain the recombinant enzyme 12S-LOX cDNA from RINm5F cells was cloned into the pPICZ, and the native stop codon prevented formation of a C-terminal His-tagged fusion protein. The plasmid was linearized and inserted into competent *P. pastoris* GS115 cells. All positive clones obtained by using zeocin as a selection marker were selected and cultured in minimal medium containing the required substituents. Cell lysates were centrifuged, and the stroma-free lysate supernatant was used as enzyme source.

The crude recombinant enzyme obtained above was purified and incubated with 20 μM 12S-HpETE. As can be seen in Figs. 4A and B 12S-HpETE was predominantly converted by the enzyme to HXA_3 (trace I, measured as $TrXA_3$ derivative), and to a much smaller amount of HXB_3 (trace II), indicating a high degree of product specificity. Heat-denaturing of the recombinant enzyme exhibited no catalytic activity (trace III). In addition, application of a specific 12S-LOX inhibitor 4-(2-oxapentadeca-4-yne)phenylpropanoic acid (OPP) completely abolished HXA_3 formation [42].

HXA_3 synthase activity in 12S-LOX is dependent on the positional specificity of AA oxygenation

Although LOXs have been a subject of investigation for decades, their crystal structures have been only recently described. The first crystal structure of rabbit reticulocyte-type 15-LOX was solved just 8 years ago [46]. Herein the enzyme was presented as a two-domain protein. The N-terminal domain constitutes a β-barrel similar to that of β-barrel domain in mammalian lipases and primarily responsible for placing the enzyme near the substrate, and a large C-terminal catalytic domain containing the non-heme iron

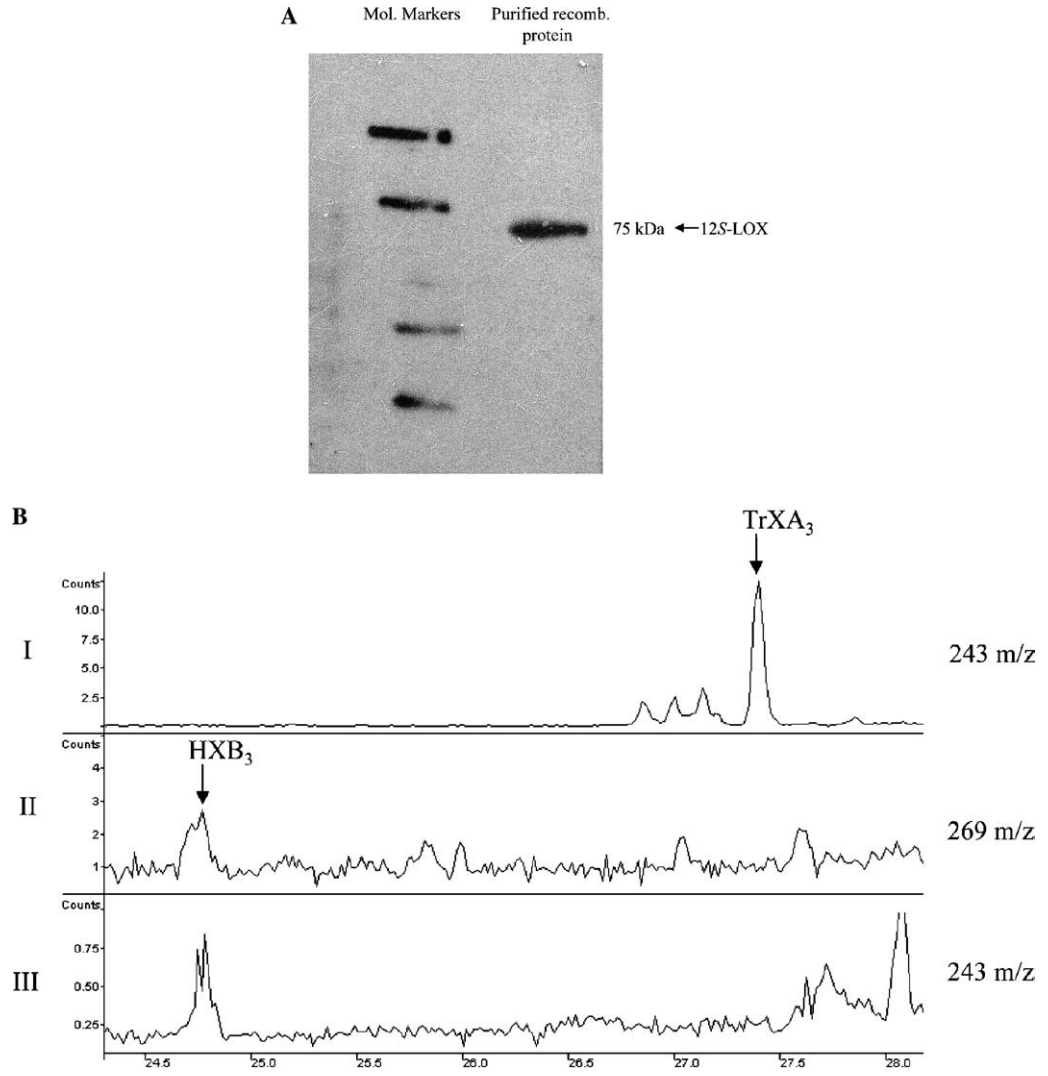

Fig. 4. (A) Western blot of a purified rat leukocyte-type 12S-LOX protein from RINm5F cells [40]; (B) mass chromatogram profile of Me-TMS derivatives of $TrXA_3$ (trace I, characteristic ion *m/z* 243), HXB_3 (trace II, characteristic ion *m/z* 269) after incubation with 20 μM 12S-HpETE of RINm5F cells. Trace III shows the mass chromatogram profile of $TrXA_3$ after incubation of 12S-HpETE with heat-denatured RINm5F cells.

atom which is coordinated by three histidine residues and the carboxy-terminal isoleucine [46], both incoherently bound to each other [46]. Based on the crystal structure of the rabbit 15-LOX, and experiments carried out with this enzyme using a number of AA isomers, it was conjectured that the oxygenation rate and the positional specificity of 15-LOXs with respect to the substrate depend on the distance of the double allylic methylene from the ω-end of the AA isomer [47]. Recent structural studies on 15-LOX however do not differentiate between 12- and 15-LOX, rather denote them as 12/15-LOX, because it is relatively easy to transform a 15-LOX in 12-LOX and vice versa.

In earlier reports it has been shown that various 12-LOX types upon incubation with AA may lead to different ratio of 12S- or 15S-LOX products, depending upon the enzyme type employed. Thus, the ratio of 12S-LOX to 15S-LOX products varies from ~3:1 for the murine leukocyte 12S-LOX to ~6:1 for the rat brain 12S-LOX to ~9:1 for the porcine leukocyte 12S-LOX, and to ~11:1 for the bovine tracheal leukocyte 12S-LOX [48]. To investigate if various LOX isoforms exhibit HXA_3 synthase activity in addition to LOX activity, our laboratory determined the conversion of 12S-HpETE to HXA_3 as well as to 12- and 15-HETE by these enzymes expressed in *Escherichia coli*. Whereas human 5S-LOX and rabbit reticulocyte 15S-LOX exhibited almost no HXA_3 synthase activity (<0.01 μg $TrXA_3$/ml culture) and ratio of 12S- to 15S-HETE being nil and 3:97, respectively, human platelet-type and rat leukocyte-type 12S-LOX exhibited 0.46 and 0.85 μg $TrXA_3$/ml culture, respectively [42]. We therefore concluded that the positional specificity of the 12S-LOX isoform may be determinant for the HXA_3 synthase activity. It has been proposed that the size and shape of the AA-binding pocket is vital for the positional specificity concerning the amino acid residues [49]. Crystal structure of the reticulocyte-type 15-LOX showed a U-shaped active site cavity lined up with side chains from Phe353, Met419, Ile418, and Ile593 amino acids. These amino acid residues correspond to those

positions of the leukocyte-type 12S-LOX, the active site cavity of which is seemingly slightly larger than that of the reticulocyte-type 15-LOX [46]. Met419 and Ile418 in reticulocyte-type 15-LOX have been identified as sequence determinants [49,50]. Consequently, their mutation to residues with smaller side chain, such as Val, yields a mutant enzyme that functions as arachidonate 12S-LOX [50]. Conversely, reverse mutation of these amino acids in porcine leukocyte-type 12S-LOX yields an enzyme which functions as reticulocyte-type 15S-LOX [51]. However, this mutation concept failed in case of leukocyte-type 12S-LOX of rats and mice [52]. Construction of chimeric LOX species and mutagenesis studies revealed that Leu353 appears to be more important for the 12S-LOX of rats and mice [49].

Site-directed mutagenesis

Based on the above-mentioned findings we performed site-directed mutagenesis studies to change the positional specificity of rat leukocyte-type 12S-LOX and rabbit reticulocyte-type 15S-LOX with respect to AA as substrate. Target amino acids were localized by performing the amino acid alignment of both rat 12S- and rabbit 15S-LOXs (Fig. 5A). Sequence determinants identified in the rabbit 15S-LOX were Phe353, Gln417, Ile418, and Ile593, whereas in rat 12S-LOX these were Leu353, Lys417, Ala418, and Val593. To document the positional specificity of the rat leukocyte-type 12S-LOX several mutant enzymes were constructed by interchanging small amino acid residues with space filling side chains, and HXA_3 synthase activity was assayed. The results obtained from these mutants (Fig. 5B) can be summarized as follows: (a) all mutant enzymes were active; (b) HXA_3 synthase activity was inherent in all 12S-LOX mutants exhibiting predominantly 12-lipoxygenation; (c) HXA_3 synthase activity in rat 12S-LOX mutant (L353F), which is converted to 15-lipoxygenating enzyme, derives from the residual 12S-LOX activity of the mutant; (d) whereas rat 12S-LOX mutant (A418I) possesses 12S-LOX and HXA_3 synthase activities, the reverse mutation of rabbit reticulocyte 15S-LOX (I418A) renders 12-lipoxygenation capacity and HXA_3 synthase activity to the enzyme. In conclusion, rat 12S-LOX possesses an intrinsic HXA_3 synthase activity, which is only impaired when the positional specificity of AA oxygenation is changed to 15-lipoxygenation.

With respect to kinetic analysis, 12S-HpETE was found to possess a lower binding affinity at the active site of rabbit 15S-LOX and its HXA_3 synthesizing mutant (I418A) than that for AA (K_m values 91.5 and 481.5 μM for 12S-HpETE vs. 20.5 and 26.0 μM for AA). This is in line with previous reports, in which hydroxylated fatty acids exhibited an impaired binding affinity at the active sites of LOXs

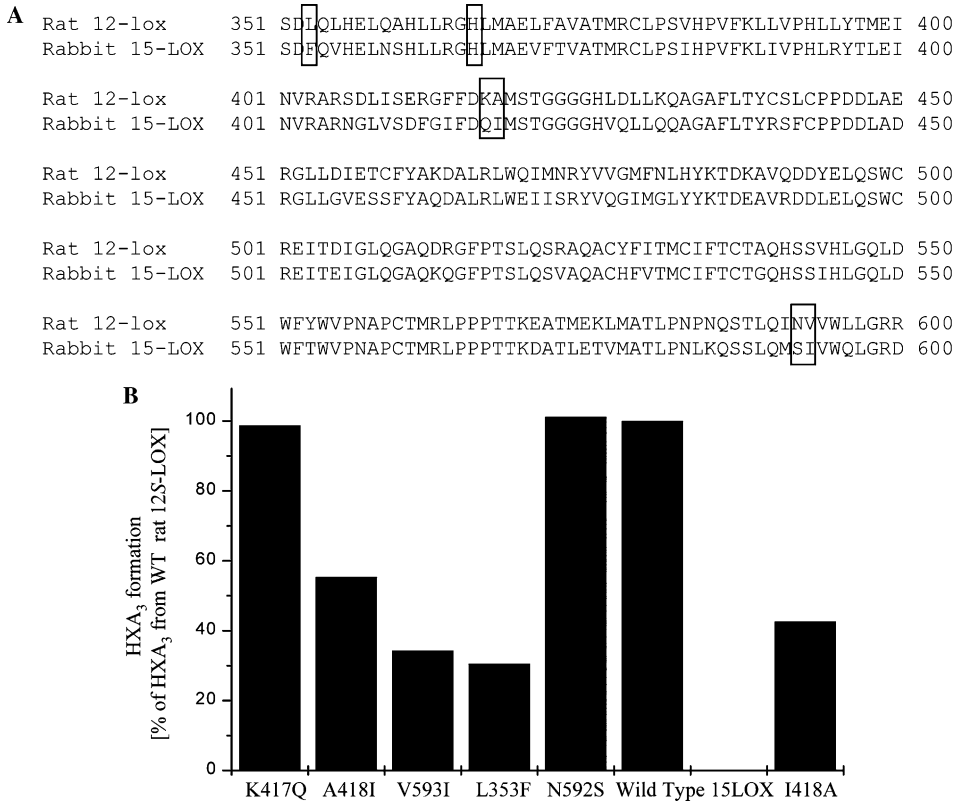

Fig. 5. (A) Amino acid alignment between the rat leukocyte-type 12S-LOX and the rabbit leukocyte-type 15S-LOX. Sequence determinants are boxed. [40]; (B) HXA_3 synthase activities of wild type and mutants of rat leukocyte-type 12S-LOX and rabbit leukocyte-type 15S-LOX. The last two columns are related to the wild type rabbit 15S-LOX and its mutant 1418A. The activities are expressed as percentage of HXA_3 from that of wild type 12S-LOX in two separate experiments [40].

[53]. With respect to the reaction rate, it was found to be higher for AA oxygenation with both enzymes than for 12S-HpETE (V_{max} values 22.2 and 6.67 s^{-1} for AA vs. 1.34 and 1.16 s^{-1} for 12S-HpETE) [42]. These data pinpoint that the catalytic efficiency (V_{max}/K_m ratio) of LOX-catalyzed metabolism of 12S-HpETE is lower than that of AA oxygenation, albeit I418A is capable of synthesizing HXA_3.

Regulation of hepoxilin A_3 synthase activity

The functional 5′-flanking sequences of arachidonate 12S-LOX genes have been reported for human and murine platelet isozymes [54–56], and porcine, murine, and rat leukocyte enzymes [56,57]. The rat 12S-LOX with intrinsic HXA_3 synthase activity [42] in this review displayed the highest degree of homology to porcine leukocyte 12S-LOX (71%) and to human 15S-LOX (75%) with less homology to human platelet 12S-LOX (59%) [52]. Analysis of *cis*-acting regulatory elements disclosed that the genes of leukocyte-type isozymes contain GC- and TATA-like boxes within ~110 bases of the initiation codon [56]. Further analysis of upstream regions revealed AP-1 (*c-jun*) and AP-2 binding sequences in different combinations depending upon the species [48,56]. Although ample knowledge on the analysis of functional promoter sequence in human 12S-LOX gene with respect to the role of Sp1 and NFκB-binding consensus sites is present [58,59], very little is known about the transcriptional activation of the rat 12S-LOX gene promoter in RINm5F cells, which exhibit intrinsic HXA_3 synthase activity. A myriad of agonists besides cytokines, such as platelet-derived growth factor in smooth muscle cells [60] and bradykinin and PAF in rat skin [61], have been shown to induce the 12S-LOX mRNA and protein, and to produce hepoxilins from AA.

Rat 12S-LOX-catalyzed HXA_3 synthesis involves a hydroperoxidase reaction, in which both homolytic cleavage of the peroxy group forming radical intermediates and formation of epoxy–hydroxy compounds proceed enzyme-controlled, yielding a much simpler product pattern. Inasmuch as recombinant rat leukocyte-type 12S-LOX converts exogenously added 12S-HpETE predominantly to HXA_3, a tight enzymatic control of the biosynthesis seems to be evident. Confirmation for mainly HXA_3 synthase activity was also achieved by monitoring the absorbance at 235 nm, indicating disappearance of 12S-HpETE and complete inhibition of the absorbance inhibition by 12S-LOX inhibitor OPP [42].

Plenty of evidence exists that AA metabolism in mammalian cells is regulated by glutathione peroxidases [39,43,44,62,63]. Among various forms of glutathione peroxidases cGPx and PHGPx are responsible for reducing the peroxide tone created by hydroperoxy fatty acids emanating from LOX- and/or cyclooxygenase-catalyzed reactions [40]. PHGPx differs from cGPx in its ability to react not only with free hydroperoxy fatty acids, but also with esterified ones in membrane phospholipids [64]. PHGPx has been shown to play a greater role in the regulation of 5S-, 12S-, and 15S-LOX pathways of AA metabolism in leukocytes and platelets [40,43,44,65]. Inasmuch as glutathione peroxidases (cGPx and PHGPx) and hepoxilin-forming catalysts, specially HXA_3 synthase, compete for the same substrate, it can be proposed that the overall activity of glutathione peroxidases, e.g., overall peroxide tone, determines the rate of HXA_3 formation. Consequently, native RINm5F cells, which are devoid of cGPx and PHGPx, produced only HXA_3 when incubated with AA. But, cells stably transfected with cGPx or transfected with PHGPx cDNA did not exhibit any HXA_3 formation [20]. That cGPx and PHGPx also down-regulate the cellular 12S-LOX activity could be demonstrated in HeLa cells overexpressing HXA_3 synthase activity, despite the presence of cGPx and PHGPx in abundance. However, when glutathione and PHGPx were inhibited by diethyl maleate, a significant formation of HXA_3 due to enhanced hydroperoxide tone was observed [20]. These findings give a clear-cut evidence for the intrinsic HXA_3 synthase activity in rat 12S-LOX enzyme, the regulation of which is finely tuned by cellular cGPx and PHGPx.

Recently, nitric oxide (NO) has been reported to be involved in the activation of rat 12S-LOX by interleukin-1β (IL-1β) [66]. This activation of iNOS was prevented by *N*-monomethyl-L-arginine (NMMA). Also inhibited by NMMA were 12S-LOX and HXA_3 synthase activities when RINm5F cells were stimulated by IL-1β [67]. Therefore, we do not rule out that 12S-LOX activation by IL-1β may be mediated by NO.

In summary, we have isolated a rat leukocyte-type 12S-LOX from RINm5F cells, which exhibits an intrinsic HXA_3 synthase activity. Nevertheless, it cannot be excluded that other proteins exhibit a similar activity. More research is required to sketch out other biosynthetic pathways for HXA_3 formation in different cell types and organs.

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