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Review

Regulation of 5-lipoxygenase enzyme activity

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Abstract

In this article, regulation of human 5-lipoxygenase enzyme activity is reviewed. First, structural properties and enzyme activities are described. This is followed by the activating factors: Ca^{2+}, membranes, ATP, and lipid hydroperoxide. Also, studies on phosphorylation of 5-lipoxygenase and nuclear localization sequences are reviewed.

Keywords: Leukotriene; Eicosanoid; Arachidonic acid

5-Lipoxygenase (5LO) catalyzes oxygenation of arachidonic acid (AA) to 5(*S*)-hydroperoxy-6-*trans*-8,11,14-9-eicosatetraenoic acid (5-HPETE), and further dehydration to the allylic epoxide leukotriene A_4, the initial reactions in leukotriene (LT) biosynthesis. 5LO is found in polymorphonuclear leukocytes (PMNL), monocytes/macrophages, mast cells, B-lymphocytes, also in dendritic cells, and in foam cells of human atherosclerotic tissue. LTA_4 is converted by LTA_4 hydrolase to the dihydroxyacid LTB_4 and by LTC_4 synthases to the glutathione conjugate LTC_4. LTs are inflammatory mediators which mediate quick cellular responses (as smooth muscle contraction, phagocyte chemotaxis, and increased vascular permeability) via G-protein coupled receptors. Effects on protein expression (i.e. [1]) may lead to more long-lasting responses. Different findings (gene knock-out, immunohistochemistry, and gene polymorphisms) now indicate a role for 5LO and LTs in atherosclerosis (see [2] for review). 5LO is not only involved in LT biosynthesis, 5-HPETE can also be further metabolized to 5-oxo-ETE [3] and to the lipoxin family of lipid mediators [4].

5LO structure, reactions catalyzed

5LO is a monomeric enzyme, mammalian 5LO cDNAs have been cloned from human, rat, mouse, and hamster (672 or 673 amino acids, more than 90% identity). A model of the 5LO structure, based on the crystal structure (1LOX) of the ferrous form of rabbit reticulocyte 15LO [5], consists of an N-terminal β-sandwich (residues 1–114) and the C-terminal catalytic domain (residues 121–673) which contains the catalytic iron, compare Fig. 1. When the 15LO structure was determined, the N-terminal β-sandwich was virtually identical to the C-terminal β-sandwich of pancreatic lipase [5,8], this similarity may extend also to 5LO. The N-terminal β-sandwich of 5LO resembles a C2 domain [6], and modelling was performed based also on the structure of the C2-like domain of *Clostridium perfringens* α-toxin (1QMD, a phospholipase C) [7]. Sequence and topology similarities between polycystin-1, lipoxygenase, and α-toxin defined the PLAT domain family, which was described as a subset within the C2 family [8–10].

Recombinant human 5LO and porcine leukocyte 5LO contain iron [11,12] and iron as well as enzyme activity was lost when 5LO was exposed to oxygen [11]. EPR showed that the iron of the isolated enzyme is ferrous, while treatment with lipid hydroperoxide

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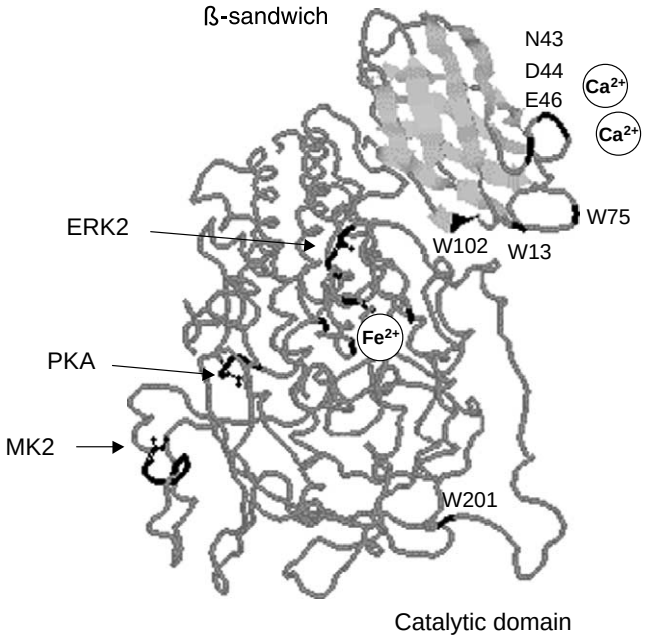

Fig. 1. Schematic model structure of 5LO, based on crystal structure for ferrous form of rabbit reticulocyte 15LO [5]. In the β-sandwich, mutagenesis of residues 43–46 reduced Ca^{2+} binding. Trp-13, -74, and -102 mediate binding to phosphatidylcholine and the stimulatory effect of OAG. In the catalytic domain, five iron ligands (His-367, His-372, His-550, Asn-554, and Ile-673) are given in black. In the three kinase motifs (MK2: LERQLS; PKA: RKSS; ERK2: YLSP), the underlined Ser residues (phosphorylated) are indicated with ball and stick. Trp-75 and Trp-201 were labelled by the ATP-analogue 2-azido-ATP. Picture generated with RasMol.

(LOOH) gave ferric iron. The multicomponent g6 signal in the EPR-spectrum indicated that several forms of the metal center were present [13]. Not only 5-HPETE, but also 15-HPETE and 13-HPODE gave EPR-detectable ferric 5LO. Similar to soybean lipoxygenase, EPR indicated a flexible iron ligand arrangement in 5LO, and selenide inhibited 5LO by binding to iron, thus abolishing the signal at g 6.2 typical of active 5LO [14]. In almost all lipoxygenases, six conserved His residues are present, with the C-termini also being very similar. Based on mutagenesis studies (reviewed in [15]) and comparisons to the crystal structures of soybean LO-1 [16,17], soybean LO-3 [18] and rabbit 15LO [5], two of the conserved His and the C-terminal Ile, appear to constitute a 2-His-1-carboxylate facial triad anchoring the iron, a common feature for active sites of mononuclear non-heme iron (II) enzymes [19]. In 5LO, the triad consists of H372, H550, and the C-terminal I673. In addition, H367 and N554 may function as replaceable ligands to iron [14,20]. Recently, we described that the function of the C-terminus as one of the iron ligands is stabilized by a hydrogen bond (Asn-699 to His-399), forming a C-terminal loop [21]. Structural specificity of lipoxygenases may depend on the space of the pocket binding the fatty acid substrate, mutagenesis reducing the pocket space converted 5LO to a 15LO [22].

In the oxygenase reaction, 5LO converts arachidonic acid to 5(*S*)-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5-HPETE). In a concerted reaction, the pro-S hydrogen is abstracted from C-7, and oxygen is inserted antarafacially. 5LO also catalyzes the next step in LT biosynthesis (5-HPETE to LTA_4), initiated by abstraction of hydrogen (pro-R at C-10). In addition to AA, other substrates for the 5LO oxygenase activity were 5,8,11,14,17-eicosapentaenoic acid, 5,8,11-eicosatrienoic acid, 5,8-eicosadienoic acid, 12-HPETE, and 15-HPETE [23–25]. Apparently, fatty acids with 5,8-*cis*-double bonds are good substrates. The activity with 8,11,14-eicosatrienoic acid [26] involves abstraction of hydrogen at C-10, as in the LTA_4-synthase reaction. At low temperature, porcine leukocyte 5LO also had a 6(*R*)-oxygenase activity, giving 5(*S*),6(*R*)-dihydroperoxy-7,9,11,14-eicosatetraenoic acid [27]. Like other lipoxygenases, 5LO also has a reducing agent dependent hydroperoxidase activity, which as the oxygenase and LTA_4-synthase activities, is stimulated by Ca^{2+} [28,29].

Factors stimulating 5LO activity via the C2-like domain: Ca^{2+} and membrane

The factors which regulate 5LO activity should result in that 5LO gets access to substrate, and to oxidation of ferrous 5LO to ferric form. Also, stabilization of an active form of 5LO may take place. Ca^{2+} together with membranes may contribute to all three effects.

Ca^{2+}

Early indications for a role of Ca^{2+} in 5LO regulation were the Ca^{2+} ionophore-activated biosynthesis of SRS-A and other 5LO products in intact cells [30,31]. The first result with a subcellular preparation was that Ca^{2+} stimulated synthesis of 5-HETE in homogenates of RBL-1 cells [32]. Ca^{2+} activates purified 5LO, however 5LO can also be active in the absence of added Ca^{2+} [26,33–36]. The EC50 for Ca^{2+} activation of purified 5LO is quite low (1–2 μM), and full activation is reached at 4–10 μM [6,37,38]. 5LO binds Ca^{2+} in a reversible manner [39], for the intact enzyme a K_d close to 6 μM was determined by equilibrium dialysis and the stoichiometry of maximum binding averaged around two Ca^{2+} per 5LO. Similar results (two Ca^{2+} per 5LO, K_{Ca} 7–9 μM) were obtained for the His-tagged C2-like domain (residues 1–115) [7]. It is of interest that both the K_d of 6 μM and the Ca^{2+} concentration required for activation in vitro are considerably higher than the intracellular Ca^{2+} concentration estimated for stimulation of 5LO in PMNL (approximately 200 nM) [40].

C2 domains typically bind Ca^{2+} via the ligand binding loops. We described that the C2-like N-terminal β-sandwich of 5LO binds Ca^{2+}, leading to Ca^{2+}

stimulation of enzyme activity [6]. Comparison to $cPLA_2$ (1RLW) suggested some candidate Ca^{2+} ligands in the four loops that were mutated [6]. The most prominent increase of Ca^{2+} concentration required for stimulation of 5LO activity was found after mutations in loop 2 (triple mutant N43A/D44A/E46A). Further modelling and comparisons to C2 (or C2-like) domains of mammalian 15LO, $cPLA_2$, and *C. perfringens* α-toxin have suggested different locations for the two bound Ca^{2+} ions [7,10,41]. Also Mg^{2+}, at concentrations which exist in cells, can activate 5LO in vitro [36] apparently via a similar site as for Ca^{2+} [6,41].

Membrane

Many C2 domains mediate Ca^{2+}-induced membrane association [42,43]. During purification of 5LO from human leukocytes, it was observed that enzyme activity depended on microsomal membranes [34,44]. Phosphatidylcholine (PC) was used as a stabilizing additive in purification of 5LO [45,46], and synthetic PC vesicles could replace the cellular membrane fraction as a stimulatory factor [47]. PC was required for both basal and Ca^{2+} stimulated 5LO activity in vitro [35,36]. It was argued that 5LO is active at a lipid–water interphase [48] performing an interfacial reaction in the same manner as phospholipase A_2 [24]. Also, treatment with Ca^{2+} caused association of 5LO with synthetic PC liposomes [38], and Ca^{2+} (as well as Mg^{2+}) increased the hydrophobicity of 5LO in a phase partition assay [36,39]. In the presence of Ca^{2+}, the isolated 5LO C2-like β-barrel had a higher affinity for zwitterionic PC vesicles than for anionic PS and phosphatidylglycerol vesicles, and three mediating Trp residues (Trp-13, -75, and -102) in the ligand binding loops were identified [7]. It was suggested that the PC selectivity accounts for the targeting of 5LO to the nuclear envelope [7], in accordance with the requirement of the β-sandwich for translocation of GFP-5LO constructs to the nuclear membrane in ionophore stimulated HEK 293 cells [49]. For the other C2 domain with PC selectivity ($cPLA_2$), Ca^{2+} is judged to promote PC membrane association in two ways, by local charge neutralization of the anionic protein surface, and by changing side-chain orientations of aliphatic and aromatic residues in the Ca^{2+} binding loops, thus leading to their membrane insertion and hydrophobic interactions. It was concluded that Ca^{2+} has the same effects on the 5LO C2-like domain [7].

Recent studies have confirmed Ca^{2+}-induced binding of intact 5LO to zwitterionic PC leading to active 5LO, and it was found that this association stabilizes the structures of both 5LO protein and membrane [50]. It was also found that 5LO can bind to other phospholipids, including cationic species. This binding was stronger and occurred in the absence of Ca^{2+}, but Ca^{2+} was required for 5LO activity. Based on these comparisons, it was suggested that 5LO can bind to membranes in "productive/non-productive modes," i.e., membrane binding per se may not confer 5LO activity. It was also found that an increased membrane fluidity favored 5LO association, 1-palmitoyl-2-arachidonoyl-*sn*-glycero-phosphocholine gave the best penetration and binding. It was argued that this should be the factor directing 5LO to the nuclear envelope, which is enriched in AA containing lipids, see [51] and references therein. Interestingly, addition of cholesterol (20 mol%) to a membrane preparation (1-palmitoyl-2-oleoyl-*sn*-glycero-phosphocholine) reduced 5LO activity by half [51]. As $cPLA_2$ (containing C2 and catalytic domains) [52,53], and in similarity to 15-lipoxygenase [54], it appears reasonable that also for 5LO residues in the catalytic domain contribute to membrane association.

Another type of lipid, various glycerols, was recently found to stimulate 5LO. Of the compounds tested, OAG (1-oleoyl-2-acetyl- *sn*-glycerol) was most potent, but also OG (1-*O*-oleyl-rac-glycerol), DOG (1,2-dioctanoyl-*sn*-glycerol), and EAG (1-*O*-hexadecyl-2-acetyl-*sn*-glycerol) were effective [55]. This occurred in the absence of Ca^{2+}, in fact Ca^{2+} prevented this stimulatory effect of OAG. Also, phospholipids or cellular membranes abolished the effects of OAG. Intriguingly, the three Trp residues which mediate binding of the 5LO C2-like domain to phospholipids (Trp-13, -75, and -102) [7] were involved, while the mutant 5LO-W13A/W75A/W102A was not stimulated by OAG. It was concluded that OAG directly stimulates 5LO by acting at the phospholipid-binding site located within the C2-like domain. Apparently, the uncharged glycerols can bind to the C2-like domain of 5LO in the absence of charge neutralization, or the changes in side chain orientations, induced by Ca^{2+}.

Activation by ATP

ATP was first shown to stimulate crude 5LO [56]. There was no effect of ATP alone, but in the presence of Ca^{2+} K_a values for ATP were 30–100 μM [23,33,57]. The stimulatory effect has been confirmed with purified enzymes and also other nucleotides were effective [56,58] including non-hydrolyzable γ-S-ATP [59]. For purified 5LO, the effect of ATP does not depend on Ca^{2+}, about 0.1 mM ATP gave maximum activation [35,59]. In the cell, most of the ATP binds Mg^{2+}, and it appeared that a $MgATP^{2-}$ complex could replace ATP as 5LO activating factor [36]. The affinity to ATP was used for purification of 5LO from RBL-1 cells and rat neutrophils [57,60], subsequently also of recombinant 5LO, see, for example [61]. Reactive ATP-analogues bound to 5LO with stoichiometry close to 1:1, and 2-azido-ATP covalently modified Trp-75 and Trp-201, indicating that these residues were close to the reactive moiety of the

analogue, sitting on the 5LO nucleotide-binding site [61]. Located on the tip of one of the solvent exposed loops of the β-sandwich (see Fig. 1) Trp-75 is one of the three Trp residues involved in membrane association of the human 5LO [7]. This residue could be mutated without clear changes in activity and ATP-affinity. On the other hand, the mutant 5LO-W201R was barely expressed, and exchange of Trp-201 to Ala or Ser resulted in severely reduced activity and decreased ATP-column yield. In addition to being close to (or part of) an ATP-binding site, Trp-201 appears to be important for the overall structure of 5LO [21]. ATP may activate 5LO by stabilizing the protein structure. Hydrolysis of ATP is not required for the activating effect, and it appears that purified 5LO is more stable in the presence of ATP.

Activation by lipid hydroperoxide

For lipoxygenase catalysis, the ferrous iron of the resting form of the enzyme should be oxidized to ferric, by lipid hydroperoxide (LOOH). Leukocyte 5LO in crude homogenates was stimulated by 15-HPETE, 12-HPETE, 5-HPETE, or 13-HPODE, but not by cumene hydroperoxide, *tert*-butyl hydroperoxide, or H_2O_2 [62]. Accordingly, not only 5-HPETE, but also 15-HPETE and 13-HPODE gave EPR-detectable ferric 5LO [14]. Glutathione peroxidase-1 (GPx-1) added to in vitro assays of 5LO inhibited product formation [63–65] and was also found to prevent inactivation of purified 5LO [66]. Peculiarly, these effects of GPx-1 on 5LO were better supported by β-mercaptoethanol than by GSH [65,66]. The compounds Dnp-Cl and diamide stimulated 5LO in human PMNL, and for Dnp-Cl this was explained by the impairment of reduction of organic peroxides [67,68]. Subsequently, both GPx-1 and phospholipid hydroperoxide peroxidase (GPx-4) have been shown to influence cellular 5LO activity [65,69–73]. An intriguing finding was that 5LO prepared from granulocytes was more resistant to peroxidases than 5LO from the B-lymphocyte cell line BL41-E95A [65,70]. For the lymphocyte cell line BL41-E95-A, there was actually no 5LO activity of the intact cells, unless Dnp-Cl, diamide, or H_2O_2 was added to the cells [74], and in this B-cell line 5LO activity was strongly enhanced by pro-oxidizing conditions [75]. Interestingly, oxidative stress induced by diamide (a tyrosine phosphatase inhibitor) or H_2O_2 stimulated p38 MAP kinase in BL41 cells and may thus stimulate 5LO in two ways, by inducing phosphorylation (compare below) and by promoting formation of the ferric form of the lipoxygenase [76]. The efficiency of non-redox type 5LO inhibitors depended on the presence of intact GPx activity leading to low hydroperoxide concentration [77]. For two compounds (ZM 230487, L-739.010), this appeared related to oxidative stress and activation of p38 MAP kinase [78], but for the novel non-redox 5LO inhibitor CJ-13,610 this was not the case [79].

Ca^{2+} promotes 5LO β-sandwich membrane association, but it appears that Ca^{2+} also has another effect. Thus, in the presence of Ca^{2+}, Gpx-1 inhibited 5LO to a lesser degree, and this effect of Ca^{2+} was diminished for a 5LO mutant with decreased Ca^{2+} affinity (exhanges in ligand binding loop 2, N43A/D44A/E46A, compare above) [80]. It was suggested that Ca^{2+} may increase the affinity of 5LO to LOOH, thus a lower concentration of LOOH may be sufficient for the formation of ferric 5LO, when Ca^{2+} is present. Lipoxygenases may have two fatty acid-binding sites, one catalytic and one regulatory [33,81], possibly LOOH binds to a regulatory site [80].

Phosphorylation of 5LO

5LO can be phosphorylated on three residues: Ser-271, by MAPKAP kinase 2 [82]; Ser-663 by ERK2 [83]; Ser-523 by PKA catalytic subunit [84]. An early indication that kinases could influence cellular 5LO activity was the correlation of PKC and 5LO activities in alveolar and peritoneal macrophages [85]. Also, phosphorylated 5LO could be immunoprecipitated from a subcellular fraction containing nuclear associated proteins, from HL-60 cells labelled with [^{32}P]orthophosphate and then stimulated with ionophore A23187 [86]. Inhibitors of tyrosine kinases and a mitogen-activated protein kinase kinase (MKK) reduced 5LO activity in neutrophils and HL-60 cells, and it was suggested that 5LO phosphorylation could influence binding to other proteins [86,87].

p38 mitogen-activated protein kinase (p38 MAP kinase) exists in several isoforms, which are activated by cell stress or treatment of cells with inflammatory cytokines [88]. Activated p38 MAKP in turn phosphorylates and activates MAPKAP kinases 2 and 3 (MK2/3). By in-gel kinase assays, 5LO was found to be a substrate for MK2/3, these 5LO kinases being activated upon stimulation of MM6 cells, PMNL, and B-lymphocytes [76,82]. Mutation of Ser-271 to Ala in 5LO abolished MK2 catalyzed phosphorylation in vitro. Also, phosphorylation by kinases prepared from stimulated PMNL and MM6 cells was clearly reduced, indicating that this is a major site for cellular phosphorylation of 5LO [89]. Compared to the established MK2 substrate heat shock protein 27, 5LO was only weakly phosphorylated in vitro by MK2. However, addition of unsaturated fatty acids (UFAs, AA or oleic acid) upregulated phosphorylation of 5LO by active MK2 in vitro. Possibly, binding of UFAs to a putative regulatory fatty acid-binding site on 5LO [33,81] stimulated phosphorylation by MK2.

Cell-stress, which activates p38 MAPK, was found to induce 5LO product formation in leukocytes. Sodium arsenite (chemical stress) was the most efficient MK2/3 stimulus in a B-lymphocyte cell line and in human PMNL, and arsenite dose–responses for activation of p38 MAPK and 5LO coincided [76,90]. Also other stress stimuli (osmotic stress, heat shock) activated p38 MAPK and stimulated 5LO activity in human PMNL; interestingly, sodium arsenite and osmotic stress were effective also after chelation of Ca^{2+} [90]. Results supporting that MK2/3 catalyzed phosphorylation of 5LO is an alternative pathway for stimulation of 5LO in stress-stimulated leukocytes, which is different in character from Ca^{2+} activation of 5LO in ionophore-treated cells [76,78,90–92], are given in Table 1.

The different sensitivity to non-redox 5LO inhibitors is interesting, since these compounds (ZM230487) reduced acute inflammatory responses but failed to inhibit chronic inflammatory processes, see references in [78]. A site for chronic inflammation in which oxidative cell stress occurs, and in which a role for LTs and 5LO was recently demonstrated is the atherosclerotic vessel wall. Possibly, 5LO phosphorylation could be relevant at such a locus. Another example is allergic asthma, where antigen–antibody complexes activate p38 MAPK via the Fcε RI receptor, and antigen-induced LTC_4 production in sensitized mouse bone-marrow derived mast cells was inhibited 67% by SB 203580 [88]. In exercise-induced asthma hyperosmolarity of the tissues can provoke airway narrowing in asthmatics [93]. Hyperosmolarity leads to osmotic stress, possibly leading to activation of 5LO and LT biosynthesis. Other conditions in which p38 MAPK is activated, and in which LTs are implied, are ischemia–reperfusion injury, and adult respiratory distress syndrome (ARDS).

Another MAP kinase (ERK2) was found to phosphorylate 5LO in vitro, on Ser-663. Also this phosphorylation was stimulated by UFAs [83]. Phosphorylation of this site seem related to PMA-primed 5LO activity in Mono Mac 6 cells. Without PMA-priming there was actually no translocation of 5LO to the nucleus when MM6 cells were stimulated with ionophore [83,94]. For PMNL, it appeared that both ERK2 and p38 MAP kinase activities were important for AA-induced 5LO product formation (no ionophore) [83,92,95]. In PMNL depleted of adenosine (compare below, phosphorylation by PKA), AA at low concentration gave 5LO product formation [96]. It was suggested that first, already membrane bound 5LO, catalyzes an initial burst of LTB_4 biosynthesis in the absence of Ca^{2+} mobilization. Then, via an autocrine loop, LTB_4 causes the release of Ca^{2+} from intracellular stores and further LT biosynthesis. One could visualize that AA-induced phosphorylation of 5LO at Ser residues 271 and 663 could contribute to the initial activation of 5LO. ERK2 inhibitors reduced nuclear localization of 5LO in fMLP-stimulated PMNL [97], and in transfected 3T3 cells the mutant 5LO-S271A displayed reduced nuclear localization as well as LTB_4 biosynthesis (induced by ionophore plus AA) [98].

Table 1
Different features between cell stress and Ca^{2+}-induced activation of 5LO in PMNL

Feature	Cell stress	Ionophore
Ca^{2+} dependency	None	High
Decreased K_m for arachidonic acid	No	Yes
Sensitivity to p38 MAPK inhibitor SB203580	High	Low
Time-course of activity	Prolonged	Quick
Sensitivity to certain non-redox 5LO inhibitors	Low	High

Recently, a mechanism for the attenuation of LT biosynthesis by cAMP was described, PKA catalytic subunit phosphorylates 5LO on Ser-523 [84]. When 3T3 cells were cotransfected with a 5LO plasmid plus increasing amounts of a plasmid expressing PKA catalytic subunit, subsequent LTB_4 synthesis (induced by ionophore and AA) was reduced dose-dependently. For the 5LO-S523A mutant, there was no effect. Additional experiments supported that this phosphorylation gave a direct reduction of 5LO catalytic activity, possibly via allosteric changes close to the active site. Although Ser-523 is part of a NLS (compare below), the inhibitory effect was not due to changes in the cellular localization of 5LO. Inhibition of cellular LT biosynthesis by cAMP has been known for since long [99,100] and confirmed in many studies, see, e.g. [95,96]. It was suggested that PKA activation in response to agents as adenosine, PGE_2, and β-adrenergic agonists, is a mechanism for control LT biosynthesis which may be important to limit inflammation [84,95].

Nuclear localization sequences (NLS)

Upon cell activation leading to LT biosynthesis, 5LO (as $cPLA_2$) migrates to the nuclear membrane, this involves Ca^{2+}-induced binding of the C2-like domain to phospholipids. The nuclear envelope was described as a LT biosynthetic metabolon [101]. 5LO can also be imported into the nucleus [102–106]. Different patterns have been observed, particularly for peripheral blood leukocytes as compared to alveolar macrophages [107]. In blood PMNL, 5LO resided in the cytosol until the cell was activated to produce leukotrienes and then 5LO bound to nuclear membrane. In alveolar macrophages, a large part of 5LO was soluble inside the nucleus already when cells were isolated, in a location somehow associated with euchromatin. Upon activation to produce leukotrienes, the intranuclear pool of 5LO (but less so the cytosolic pool of 5LO) was bound to

the nuclear membrane [105,107]. The function of intranuclear 5LO is elusive, but it appeared that import into the nucleus (glycogen-elicited rabbit peritoneal neutrophils, human PMNs adhered to a glass surface) increased the capacity of the cell for subsequent ionophore-induced LT biosynthesis [108]. This concept was further substantiated in studies of 3T3 cells transformed with 5LO mutants (NLSs, Ser-271) [98]. Thus, activation of 5LO in the cell involves translocations of 5LO between cytosol and nucleus, and to the nuclear membrane.

Nuclear localization sequences are typically rich in basic residues. They are recognized by importins/karyopherins, which facilitate docking with the nuclear pore complex, allowing entry into the nucleus [109]. Several NLS candidates in 5LO have been described [110–112], and recently attention is focused on three sequences: Leu-111 to Asp-121; Asp-156 to Asp-166; Val-514 to Leu-535 [109,113,114]. It appeared that these NLS can act together, allowing for a graded tendency of 5LO to enter the nucleus. It was suggested that this may explain why some (but not all) macrophages in a rat lung sample had 5LO associated to the nucleus [109]. Not only import, but also regulated export from the nucleus may contribute to the distribution of 5LO, and thus its potential for LT biosynthesis [112].

Concluding remarks

Regulation of 5LO is quite similar to that of $cPLA_2$, for both enzymes Ca^{2+}-induced membrane association and phosphorylation by MAP kinases seem to be major upregulating principles. Also, both enzymes come together at the nuclear envelope of the activated leukocyte, where $cPLA_2$ (possibly assisted by FLAP) provides 5LO with free fatty acid substrate, the model for LT biosynthesis at the nuclear membrane is well established. However, 5LO may be active also at other sites, in BL41-E95-A cells formation of 5LO products from exogenous AA was considerable in the absence of detectable association with the nucleus [76], and for eosinophils cytoplasmic lipid bodies were suggested as a locus for eicosanoid biosynthesis [115]. At non-nuclear sites, 5LO should depend on transcellular supply of AA [116] or that AA is provided from another part of the cell. In either case, the S100A8/A9 complex which can bind AA may be relevant. This, in PMNL very abundant, complex was suggested to function as an intermediate AA reservoir, which may deliver AA to metabolizing enzymes hours after the phospholipid hydrolysis [117].

Phosphorylation of 5LO by MAP kinases appears to confer activation in intact cells. For $cPLA_2$ it was concluded that the C2 domain, together with another region of the protein (subjected to phosphorylation), both contributed to membrane binding. Thus, phosphorylation may prime for activity also after a stimulus (like serum-treated zymosan) which gives only a moderate transient Ca^{2+} mobilization [52,53]. Similar mechanisms may apply to 5LO, and it appears reasonable that MAPK mediated activation of 5LO could function independently, or in concert with Ca^{2+}-dependent mechanisms, for activation of 5LO under physiological conditions as well as in inflammatory disease.

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