CMLS, Cell. Mol. Life Sci. 59 (2002) 742–753
1420-682X/02/050742-12 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Enzymes and receptors in the leukotriene cascade

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Abstract. Leukotrienes are a family of paracrine hormones derived from the oxidative metabolism of arachidonic acid. These lipid mediators are recognized as important signal molecules in a variety of inflammatory and allergic conditions affecting the skin, joints, gastrointestinal and respiratory systems, in particular asthma. Such conditions are typified by local pain, tissue edema, hyperemia and functional losses. In the tissues, immunocompetent cells accumulate at the site of injury which contribute to tissue damage and perpetuation of the disease process. Leukotrienes can elicit most, if not all, of these signs and symptoms. Thus, leukotriene B_4 is one of the most powerful chemotactic agents known to date and participates in the recruitment of leukocytes. The cysteinyl leukotrienes, on the other hand, contract smooth muscles, particularly in the peripheral airways and microcirculation. Recently, drugs which block the formation and action of leukotrienes have been introduced as novel antiasthmatic medications. This chapter reviews the biochemistry, molecular biology and cell biology of the key enzymes and cognate receptors in the leukotriene cascade.

Key words. Leukotriene B_4; leukotriene C_4; cytosolic phospholipase A_2; 5-lipoxygenase; five lipoxygenase activating protein (FLAP); leukotriene A_4 hydrolase; leukotriene C_4 synthase; leukotriene receptor.

Introduction

More than 20 years have elapsed since the structural elucidation of the leukotrienes (LTs) [1, 2]. This group of bioactive lipids is derived from the metabolism of polyunsaturated fatty acids, particularly arachidonic acid [3]. In two consecutive reactions, catalyzed by 5-lipoxygenase (5-LO), arachidonic acid is transformed into LTA_4, the key intermediate in leukotriene biosynthesis (fig. 1). This highly unstable epoxide may either undergo enzymatic hydrolysis into the dihydroxy acid LTB_4 or be conjugated with glutathione to form LTC_4. The latter compound, together with its metabolites LTD_4 and LTE_4, is referred to as a cysteinyl-containing leukotriene (cys-LT).

As indicated by the name leukotriene, these compounds were originally isolated from leukocytes [1, 2]. Bone-marrow-derived cells are the main producers, particularly polymorphonuclear leukocytes, monocytes as well as tissue-bound macrophages and mast cells. Certain cells, e.g. eosinophils, basophils and mast cells, mainly synthe-

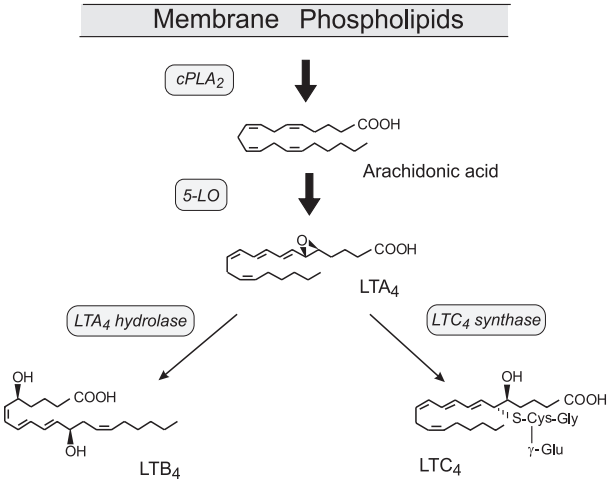

Figure 1. Key enzymes, substrates and products in the leukotriene pathway.

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size cysteinyl-containing leukotrienes, whereas others, e.g. neutrophils and macrophages, predominantly generate LTB_4.

Leukotrienes possess a wide range of biological activities elicited via specific, G-protein-coupled, cell surface receptors. Thus, LTB_4 is a very potent chemoattractant for neutrophils and recruits inflammatory cells to the site of injury. This compound also induces chemokinesis and increases leukocyte adhesion to the endothelial cells of the vessel wall. The cys-LTs are potent constrictors of smooth muscle, particularly in the airways where they elicit bronchoconstriction. In the microcirculation, they evoke constriction of arterioles and increase the permeability of the postcapillary venules, leading to extravasation of plasma. Due to their potent biological activities, leukotrienes are strong candidates as chemical mediators in a number of inflammatory and allergic disorders, e.g. rheumatoid arthritis, inflammatory bowel disease and bronchial asthma [4]. In fact, several useful drugs have been developed which block the synthesis and action of leukotrienes [5]. Some of these agents, in particular the $CysLT_1$-receptor antagonists Montelukast (Singulair) and Zafirlukast (Accolate) are presently marketed worldwide as novel antiasthmatic medications.

Cytosolic phospholipase A_2

The majority of arachidonic acid in the cell is esterified in the *sn*-2 position of phospholipids. To increase the levels of free arachidonic acid available for leukotriene biosynthesis, a phospholipase is required to release the fatty acid. Phospholipases comprise a large family of enzymes [6], several of which have been implicated in leukotriene biosynthesis. It is now well established that the group IV, high molecular weight, cytosolic phospholipase A_2 ($cPLA_2$), plays a key role in the generation of arachidonic acid for leukotriene biosynthesis. This enzyme has a molecular mass of 85 kDa, becomes activated and translocates to membranes in response to submicromolar levels of Ca^{2+}, and selectively hydrolyzes arachidonic acid esterified in the *sn*-2 position of phospholipids (fig. 2). Cytosolic PLA_2 was originally purified from U937 cells, and molecular cloning revealed a complementary DNA (cDNA) which predicts a protein of 748 amino acids (initial Met excluded) [7–9]. A protein domain, 140 amino acids long and located in the *N*-terminal part of $cPLA_2$, was shown to associate with membrane vesicles in response to Ca^{2+}. This domain contains a segment of 45 amino acids with homology to the constant region 2 of protein kinase C (PKC), believed to be of importance for translocation of PKC. Thus, $cPLA_2$, in its amino terminal part, contained a putative Ca^{2+}-dependent phospholipid binding (CaLB) domain. Experiments with recombinant DNA technology verified the regulatory

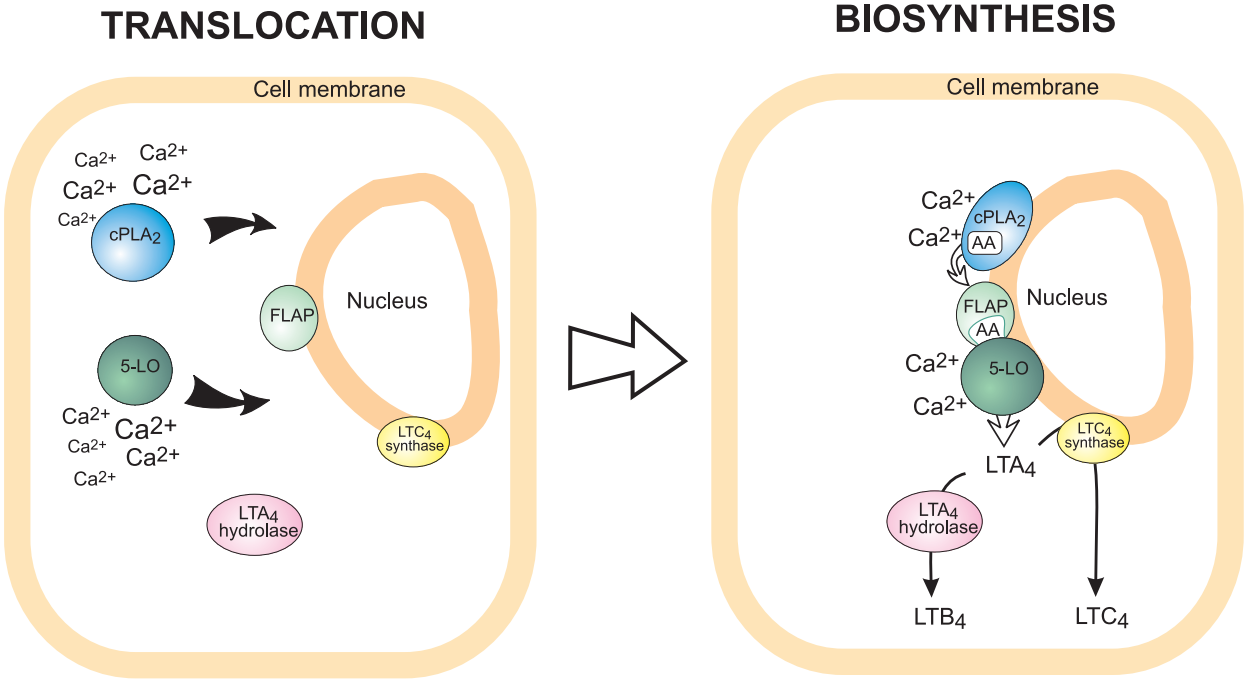

Figure 2. Translocation of $cPLA_2$ and 5-LO in a stimulated leukocyte followed by leukotriene biosynthesis at the nuclear membrane. For details, see text.

Table 1. Properties of enzymes and receptors in leukotriene biosynthesis and action*.

Protein	Protein size (no of amino acids)[a]	Prosthetic group[b]	Gene size (kb)	Exon no.	Putative *cis* elements of promoter regions	Chromosomal location	Gene-deficient mice
$cPLA_2$	748	–	–	–	CA repeats, polypyrimidine tract, AP-1, GRE	1	+
5-lipoxygenase	673	Fe	>82	14	Sp1, AP-2, NF-κB	10	+
FLAP	160	–	>31	5	TATA, AP-2, GRE	13	+
LTA_4 hydrolase	610	Zn	>35	19	XRE, AP-2	12	+
LTC_4 synthase	149	–	2.5	5	Sp1, AP-1, AP-2	5	+
BLT_1	351	–	5.5	3	Sp1, CpG site, NFκB, AP-1	14	+
BLT_2[c]	357	–	ND[c]	1[c]		14	–
$CysLT_1$	336	–	ND	ND		X	–
$CysLT_2$	345	–	ND	ND		13	–

* Data refer to human proteins. ND, not determined.
[a] Initial methionine excluded.
[b] 1 mol metal per mol protein.
[c] The ORF of BLT2 is included in the promoter of the BLT1 gene.

role of this protein segment for the Ca^{2+}-dependent binding of phospholipids, and also demonstrated that the catalytic center was localized in the remaining *C*-terminal portion of the polypeptide chain [10]. Recently, the solution structure of the C2 domain of $cPLA_2$ demonstrated that it is a β-sandwich structure that binds two Ca^{2+} ions and preferentially interacts with phosphocholine headgroups [11]. The Ca^{2+}-dependent translocation of $cPLA_2$ to endoplasmic reticulum (ER) and nuclear membranes with concomitant enzyme activation [12, 13] requires a functional C2 domain [14]. In addition, the complete crystal structure of $cPLA_2$ at 2.5 Å resolution has been determined and revealed an unusual catalytic Ser-Asp dyad, located in a deep cleft at the center of a hydrophobic funnel, which cleaves arachidonoyl phospholipids [15]. Interestingly, the structure also revealed a flexible lid that must move to allow substrate access to the active site. Cytosolic PLA_2 is also regulated posttranslationally by phosphorylation, particularly at Ser-505, which leads to activation in the presence of Ca^{2+} [16, 17].

Cytosolic PLA_2 has also been shown to bind with high affinity and specificity to phosphatidylinositol 4,5-bisphosphate (PIP_2) in a 1:1 stoichiometry [18]. This interaction results in a dramatic increase in catalytic activity, and is probably mediated via binding of PIP_2 to a putative pleckstrin homology domain in $cPLA_2$, a structural motif that is also present in phospholipase Cδ_1.

The promoter of the human $cPLA_2$ gene has been cloned and partially characterized [19]. It has features typical of a housekeeping gene with no TATA or CAAT box, although atypical in that it is not GC rich and lacks Sp1/Egr-1 sites. Instead, it has a long stretch of CA repeats and a polypyrimidine tract. The 5′-flanking region also contains a putative composite AP-1 site and glukocorticoid response element (GRE). See also table 1.

Since most of the ligands that have been used to activate $cPLA_2$ are not related to leukotriene biosynthesis, the role of $cPLA_2$ in this process has been under debate. However, mice lacking the $cPLA_2$ gene have recently been generated by targeted gene disruption, and interestingly, the peritoneal macrophages of the $cPLA_2$-deficient mice were unable to generate either prostaglandins or leukotrienes in response to adequate stimuli [20, 21]. Hence, $cPLA_2$ is certainly a critical lipase involved in leukotriene biosynthesis.

5-LO

The first two steps in leukotriene biosynthesis are catalyzed by 5-LO [22, 23]. Free arachidonic acid is oxygenated into the hydroperoxide 5-hydroperoxy-8,11,14-*cis*-6-*trans*-eicosatetraenoic acid (5-HPETE), which is subsequently dehydrated to yield the unstable epoxide intermediate LTA_4. For maximal activity, 5-LO requires both Ca^{2+} and ATP and is stimulated by lipid hydroperoxides and phosphatidylcholine [24–26]. 5-Lipoxygenase is a soluble monomeric enzyme with a molecular mass of about 78 kDa [25] and contains one atom of non-heme iron, believed to be of importance for catalysis [27]. The cDNA encoding human 5-LO has been cloned, and the deduced amino acid sequence revealed the presence of six histidines, canonical in plant and animal lipoxygenases [28, 29]. Mutagenetic analysis has demonstrated that His-372 and His-550 are iron ligands, whereas the iron-binding function of His-367 remains uncertain

[30–34]. From the three-dimensional structure of soybean lipoxygenase-1 [35, 36], it was discovered that the *C*-terminal isoleucin, Ile-673 in human 5-LO, also functions as an iron ligand [37]. Two other amino acids in 5-LO, namely Glu-376 and Gln-558 are functionally important, since mutagenetic replacements of these residues resulted in complete loss of enzyme activity [31, 32].

The crystal structure of a mammalian lipoxygenase, namely rabbit 15-LO, has been determined [38]. Interestingly, the architecture of the substrate binding site clarified the molecular mechanism for the positional specificity for oxygenation of arachidonic acid displayed by 5-, 12- and 15-LOs. Thus, the depth and width of the substrate binding pocket appears to be critical such that a shallow pocket puts the catalytic iron close to C15, whereas a deep pocket puts it close to C5. Furthermore, rabbit 15-LO contained an *N*-terminal so-called β-barrel domain, a structure also found in the *C*-terminal domain of lipases. The role of this domain for lipoxygenases is presently unclear, but for 5-LO it has been shown to bind Ca^{2+} and presumably facilitates its association of 5-LO with membranes during catalysis [39]. This β-barrel domain has also been shown to be essential for translocation to the nuclear membrane, a typical feature of 5-LO [40]. Moreover, 5-LO is also a substrate for p38 kinase-dependent mitogen-activated protein kinase-activated protein kinase (MAPKAP) kinases in vitro, suggesting that phosphorylation may be one additional factor, which determines 5-LO translocation and enzyme activity [41] (cf. fig. 2).

The gene encoding human 5-LO is located on chromosome 10 and spans more than 80 kb of DNA consisting of 14 exons divided by 13 introns [42, 43]. The promoter region lacks a typical TATA or CCAT box but contains a number of GC boxes, potential binding sites for the transcription factors Sp1 and Egr-1. Thus, the promoter structure resembles those of so-called housekeeping genes, which are constitutively expressed in many cells and tissues. This was a surprising finding since 5-LO activity has been detected almost exclusively in bone marrow-derived cells. The promoter of the 5-LO gene has been found to contain naturally occurring mutations with respect to the number of GC boxes, which in turn appears to influence the expression of the 5-LO gene, as judged from promoter-reporter activities in cell culture [44]. It was suggested that this family of polymorphisms could be related to the interindividual differences in clinical effects of 5-LO inhibitors that are observed among asthma patients. In fact, a pharmacogenetic association between 5-LO promoter genotype and the response to antiasthma treatment was recently demonstrated [45].

The role of 5-LO and its products has been studied by gene targeting [46]. 5-LO-deficient mice are more resistant to lethal effects of shock induced by platelet-activating factor (PAF) and also show a marked reduction in the ear inflammatory response to exogenous arachidonic acid but not to phorbol ester. Interestingly, the inflammatory response induced by arachidonic acid could be virtually eliminated by the prostaglandin synthase inhibitor indomethacin in 5-LO-deficient mice, but not in normal animals, suggesting links between prostaglandins and 5-LO pathways during inflammatory reactions [47]. It has also been shown that 5-LO null mice are more susceptible to infections with *Klebsiella pnemoniae*, in line with a role for 5-LO and its products in antimicrobial host defense [48]. Moreover, 5-LO deficient mice exhibit a reduced airway reactivity in response to methacholine and lower levels of serum immunoglobulins [49].

5-LO activating protein

In intact cells, 5-LO becomes activated and translocates to membranes in response to Ca^{2+}, a process accompanied by catalysis and enzyme inactivation (fig. 2) [50–52]. Cellular 5-LO activity is dependent on a small membrane protein, 5-LO-activating protein (FLAP). This protein was discovered through the inhibitory action of a drug, MK-886, on leukotriene biosynthesis in intact cells [53]. MK-886 binds to this membrane protein and can thereby prevent and revert translocation of 5-LO. FLAP was purified by means of affinity chromatography, characterized and cloned [54, 55]. The amino acid sequence showed that it is a unique protein with three putative transmembrane domains. Experiments with osteosarcoma cells transfected with 5-LO cDNA alone or together with FLAP cDNA demonstrated that FLAP is essential for leukotriene biosynthesis in intact cells [55]. Although the mechanism of action for FLAP is not fully understood, it has been suggested that FLAP presents or transfers arachidonic acid to 5-LO. Thus, FLAP was shown to be an arachidonate binding protein and this binding could be competed by compounds such as MK-886 [56]. FLAP also stimulates the utilization of arachidonic acid by 5-LO and increases the efficiency with which 5-LO converts 5-HPETE into LTA_4 [57]. However, it seems likely that additional, yet unidentified factor(s) are required for optimal expression of cellular 5-LO activity.

The FLAP gene has been cloned and spans more than 31 kb on chromosome 13 [58, 59]. It is divided into five small exons separated by four large introns and the promoter region contains a possible TATA box as well a potential GRE and AP-2 binding site (table 1).

The role of leukotrienes as inflammatory mediators has also been corroborated in studies of FLAP-deficient mice. Like the 5-LO (–/–) mice, these animals showed a blunted response to topical arachidonic acid, had increased resistance to PAF-induced shock and responded with less edema in zymosan-induced peritonitis [60]. Furthermore, the severity of collagen-induced arthritis

was substantially reduced in FLAP (–/–) mice, indicating a role for leukotrienes in this model of inflammation [61].

Leukotriene A_4 hydrolase

Leukotriene A_4 hydrolase catalyzes the final step in the biosynthesis of the proinflammatory compound LTB_4. The protein is widely distributed and has been detected in almost all mammalian cells, organs and tissues examined [62]. Among the cellular elements of blood, neutrophils, monocytes, lymphocytes and erythrocytes are rich sources of the enzyme, whereas eosinophils have low levels and basophils and platelets seem to lack LTA_4 hydrolase. The enzyme is found even in cells lacking 5-LO activity, and thus the ability to provide the substrate LTA_4. Examples of such cells are erythrocytes, T cell lines, fibroblasts, endothelial cells, keratinocytes and airway epithelial cells [62].

LTA_4 hydrolase has been purified from several mammalian sources, and cDNAs encoding the human, mouse, rat, and guinea-pig enzymes have been cloned and sequenced [63]. They all contain 610 amino acids with a molecular mass of ~69 kDa. The organization of the human LTA_4 hydrolase gene has also been determined [64]. It spans more than 35 kb of DNA and contains 19 exons. The gene resides on chromosome 12 and the 5′-flanking region contains two xenobiotic-response elements (XREs). See also table 1.

Mice deficient in LTA_4 hydrolase have been generated by targeted gene disruption [65]. These mice develop normally and are healthy. Analysis of their reactivity against various proinflammatory stimuli revealed that LTA_4 hydrolase is required for the formation of LTB_4 during an *in vivo* inflammatory reaction. Comparing the phenotype of these mice with that of 5-LO (–/–) mice allowed a delineation of the relative contribution of LTB_4 and cys-LTs, respectively, to a specific inflammatory response. Thus, LTB_4 is responsible for the characteristic influx of neutrophils, which follows topical application of arachidonic acid and contributes to the vascular changes observed in this inflammatory model. In zymosan A-induced peritonitis, LTB_4 modulates only the cellular component of the response, whereas LTC_4 appears to be responsible for the plasma protein extravasation. Moreover, LTA_4 hydrolase was shown to be upregulated in the hearts of angiotensin II-induced hypertensive rats, thus providing further evidence for a role of LTA_4 hydrolase in inflammatory reactions in vivo [66]. Of note, LTA_4 hydrolase (–/–) mice are resistant to the lethal effects of systemic shock induced by PAF, thus identifying LTB_4 as a key mediator of this reaction.

LTA_4 hydrolase belongs to the M1 family of metallopeptidases

Sequence comparison with certain zinc metalloenzymes, e.g. aminopeptidase M and thermolysin, has revealed the presence of a zinc binding motif (**H**EXX**H**-X_{18}-**E**) in LTA_4 hydrolase [67, 68]. Accordingly, LTA_4 hydrolase was found to contain one atom of zinc, the primary function of which was catalytic (fig. 3) [69, 70]. The three proposed zinc binding ligands, His-295, His-299 and Glu-318, were verified by mutagenetic analysis [71]. Furthermore, the enzyme was found to exhibit a previously unknown peptidase activity which was selectively stimulated by monovalent anions, e. g. chloride ions, in a manner suggesting the presence of an anion binding site (fig. 3) [70, 72, 73]. The physiological substrate for the peptidase activity has not been identified, but kinetic data have indicated that LTA_4 hydrolase may be an arginine tripeptidase [74].

Based on its zinc signature, sequence homology and aminopeptidase activity, LTA_4 hydrolase has been classified as a member of the M1 family of the MA clan of metallopeptidases [75]. Thus, LTA_4 hydrolase is distantly related to many other zinc proteases and aminopeptidases that are present in a variety of organisms from bacteria to mammals, including human enzymes such as aminopeptidase A (APA), aminopeptidase B (APB), aminopeptidase N (APN) and angiotensin-converting enzyme (ACE).

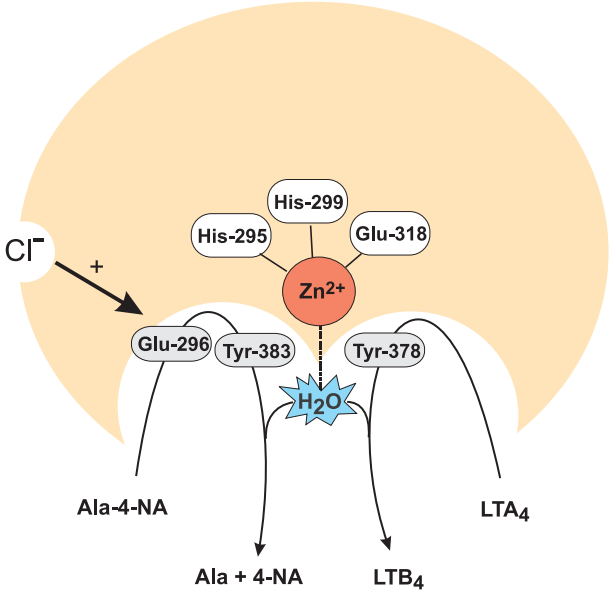

Figure 3. Model of structural and functional properties of the bifunctional, zinc-containing LTA_4 hydrolase.

Identification of catalytically important amino acid residues in LTA_4 hydrolase

In addition to the zinc-binding ligands, several amino acid residues of catalytic importance have been identified by site-directed mutagenesis. Thus, mutagenetic replacements of Glu-296 in LTA_4 hydrolase, a conserved residue within the zinc signature, abrogated only the peptidase activity and not the ability to catalyze the conversion of LTA_4 into LTB_4, which suggests a direct catalytic role for Glu-296 in the peptidase reaction, possibly as a general base [76, 77].

Furthermore, sequence comparisons and mutational analysis have demonstrated that Tyr-383 plays an important role in the peptidase reaction of LTA_4 hydrolase, presumably as a proton donor [78]. Further investigation of the catalytic properties of mutants in position 383 revealed the formation of large quantities of a novel metabolite of LTA_4 structurally identified as 5*S*,6*S*-dihydroxy-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid (5*S*,6*S*-DHETE), in addition to the expected LTB_4 [79]. Interestingly, the stereochemistry of 5*S*,6*S*-DHETE implies an S_N1 mechanism in its formation, which in turn indicates that conversion of LTA_4 into LTB_4 proceeds according to the same mechanism.

When the structure of LTA_4 hydrolase was determined (see below), a glutamic acid residue (Glu-271), belonging to a conserved G*X*MEN motif in the M1 family of zinc peptidases, was found to reside at the active site [80]. By mutational analysis and crystallography it was shown that Glu-271 is necessary for both catalytic activities of LTA_4 hydrolase [81]. Presumably, the carboxylate of the glutamic acid residue participates in the opening of the epoxide moiety of LTA_4 and formation of a carbocation intermediate. In the peptidase reaction, the role of Glu-271 may be to serve as an *N*-terminal recognition site and to stabilize the transition state during turnover of peptide substrates.

Typically, LTA_4 hydrolase undergoes 'suicide' inactivation with a concomitant covalent modification of the enzyme by its substrate LTA_4 [82]. During the inactivation process, LTA_4 binds to a 21-residue peptide located in the middle of the polypeptide chain [83]. Mutational analysis has demonstrated that Tyr-378, located within this peptide, is a major structural determinant for suicide inactivation [84]. Mutated proteins, carrying a Gln or Phe residue in position 378, were neither inactivated nor covalently modified by LTA_4. In addition, the mutated enzymes produced an isomer of LTB_4 (Δ^6-*trans*-Δ^8-*cis*-LTB_4), suggesting that Tyr-378 may also play a role in the formation of the correct double bond geometry in the enzymatic product LTB_4 [85]. Some structural and functional properties of LTA_4 hydrolase are summarized in figure 3.

The crystal structure of LTA_4 hydrolase

Recently, the X-ray crystal structure of LTA_4 hydrolase in complex with the competitive inhibitor bestatin was determined at 1.95 Å resolution [80]. The protein molecule is folded into an *N*-terminal, a catalytic and a *C*-terminal domain, packed in a flat triangular arrangement. Although the three domains pack closely and make contact with each other, a deep cleft is created between them. Interestingly, the catalytic domain is structurally very similar to the bacterial protease thermolysin, although the sequence identity is only about 7% over the corresponding polypeptide chains.

At the bottom of the interdomain cleft, the zinc site is located. As predicted from previous work [71, 86], the metal is bound to the three amino acid ligands, His-295, His-299 and Glu-318. In the structure, however, Zn^{2+} is also bound to bestatin, creating a pentavalent coordination. In the vicinity of the prosthetic zinc, the catalytic residues Glu-296 and Tyr-383 are located at positions that are commensurate with their proposed roles as general base and proton donor in the aminopeptidase reaction.

Leukotriene C_4 synthase

Leukotriene C_4 synthase catalyzes the committed step in the biosynthesis of cys-LTs through conjugation of LTA_4 with glutathione (fig. 1). The enzyme is a membrane-bound homodimer with a subunit molecular mass of 18 kDa [87–89]. It is stimulated by divalent cations, particularly Mg^{2+}, as well as phosphatidylcholine, whereas reduced glutathione is required for stability. LTC_4 synthase has been cloned and sequenced [90, 91]. Two consensus sequences for protein kinase C phosphorylation were found, and subsequent studies have shown that phosphorylation reduces the LTC_4 synthase activity [92]. Surprisingly, sequence comparisons of LTC_4 synthase and FLAP demonstrated a 31% identity between the two proteins. In addition, recent work has identified two microsomal GSH transferases (MGST2 and MGST3) which both possess LTC_4 synthase activity and exhibit a high degree of similarity to both LTC_4 synthase and FLAP [93, 94]. Sequence comparisons between various members of the LTC_4 synthase/FLAP/MGST gene family have identified conserved residues of potential functional importance, and subsequent mutational analysis suggested that Arg-51 and Tyr-93 in human LTC_4 synthase are both essential for catalysis. The guanidinium moiety of Arg-51 is believed to open the epoxide, whereas Tyr-93 acts as a base to generate a GSH thiolate anion for attack at C-6 of LTA_4 [95].

The complete gene for human LTC_4 synthase has also been cloned and sequenced [96]. This gene is located on chromosome 5, spans about 2.5 kb and has a structure similar to the FLAP gene. Thus, it contains five exons and

has exon-intron junctions that align identically with those of FLAP. The promoter region of the LTC_4 synthase gene contains several potential *cis* elements, including Sp1, AP-1 and AP-2 (table 1). Further promoter characterization revealed that an Sp1 site and a putative initiator element (Inr) are involved in non-cell-specific expression, whereas a Kruppel-like transcription factor and Sp1 are implied in cell-specific regulation of the LTC_4 synthase gene [97].

Recently, LTC_4 synthase deficient animals were generated by targeted gene disruption [98]. The LTC_4 synthase (–/–) mice developed normally and were fertile. In agreement with the results obtained with LTA_4 hydrolase (–/–) mice, the LTC_4 synthase (–/–) mice displayed a reduced plasma protein extravasation in zymosan A-induced peritoneal inflammation. In addition, these mice were less prone to develop passive cutaneous anaphylaxis. Certainly, the LTC_4 synthase deficient mice will be a useful tool for further studies of the biological role of LTC_4 in physiological as well as pathophysiological conditions. For instance, further insights may be gained regarding the recently described role of LTC_4 in the primary immune response involving mobilization of dendritic cells to lymph nodes [99], or the LTC_4 deficiency associated with pediatric neurodegenerative diseases [100, 101].

Leukotriene biosynthesis occurs at the nuclear envelope

In addition to what has been discussed above, all the enzyme components of the leukotriene cascade are regulated at multiple levels by a complex network of signaling mechanisms, e.g., cytokines, lipid mediators and transcellular metabolism [102]. It is not within the scope of this review to cover these intricate relationships; instead, we will focus on the mechanisms and consequences of the Ca^{2+}-dependent translocation of 5-LO and assembly of a biosynthetic complex at the nuclear envelope (fig. 2). Early studies showed that upon cell stimulation, 5-LO is activated, translocates to a membrane compartment and gets rapidly inactivated, in a Ca^{2+}-dependent manner [51, 52]. Experiments with mouse mast cells stimulated with ionophore A23187 or anti-IgE showed that translocation may be reversible and that the strength and duration of the Ca^{2+} influx may determine the reversibility of translocation as well as the extent of enzyme inactivation [103]. Furthermore, using the reversible 5-LO inhibitor zileuton, it was demonstrated that translocation and catalysis are not necessarily coupled and that translocation/membrane association can alter the substrate specificity of 5-LO and increase the efficiency of LTA_4 biosynthesis [104]. Of particular interest was the discovery that FLAP is localized to the nuclear envelope of resting and activated neutrophils and that 5-LO, upon cell activation, translocates to the same compartment [105]. Translocation of 5-LO also occurs in cells that do not express FLAP, and has therefore been suggested to be a FLAP-independent process [106]. Furthermore, 5-LO has been shown to associate with growth factor receptor-binding protein 2 (Grb2), an 'adaptor' protein for tyrosine kinase-mediated cell signaling, through Src homology 3 (SH3) domain interactions [107]. SH3 interactions regulate the assembly of protein complexes involved in cell signaling and cytoskeletal organization, and may form the molecular basis for 5-LO translocation and compartmentalization. In agreement with this hypothesis, inhibitors of tyrosine kinase activity, a determinant of SH3 interactions, also inhibited the catalytic activity of 5-LO and its translocation during cellular activation [108]. In addition, an internal bipartite nuclear localization sequence, spanning Arg-638 – Lys-655, has been shown to be necessary for the redistribution of 5-LO to the nuclear compartment [109, 110]. Moreover, very recent data indicate that also the *N*-terminal β-barrel domain in 5-LO plays a role in this process [40]. In this context, it is interesting to note that a variant of 5-LO, posttranslationally modified by phosphorylation, has been identified [108]. The phosphorylated 5-LO accumulated in the nuclear fraction of activated HL-60 cells, suggesting that phosphorylation may be functionally coupled to the 5-LO translocation process as well.

Further analysis of the enzyme compartmentalization revealed that 5-LO can also be present in the nucleus of resting cells associated with the nuclear euchromatin, a site from which it translocates to the nuclear envelope [111]. In polymorphonuclear neutrophils, 5-LO translocation was associated with functional responses such as activation, adherence and increased ability to synthesize LTB_4 [112]. On the other hand, adherence of eosinophils was accompanied by reduced capacity to synthesize LTC_4 [113]. This effect was explained by a resistance to activation of the nuclear pool of 5-LO. Apparently, the subcellular distribution, directional migration and activation of 5-LO in response to Ca^{2+} mobilization appear to differ between species and cell types [114]. As previously discussed, $cPLA_2$ also translocates to the nuclear membrane upon cell stimulation, and LTC_4 synthase seems to reside at the same site [12, 13, 88]. Interestingly, the soluble LTA_4 hydrolase was recently reported to reside in the nucleus of rat basophilic leukemia cells and rat alveolar macrophages, whereas rat neutrophils only contained immunoreactive protein in the cytosol [115].

Together, these findings imply that leukotriene biosynthesis is executed by a complex of enzymes assembled at the nuclear membrane (cf. fig. 2). This conclusion in turn suggests that these enzymes and their products may have additional intracellular and intranuclear functions, perhaps related to signal transduction or gene regulation. In line with this notion, it has been reported that LTB_4 is a

natural ligand to the nuclear orphan receptor PPARα, suggesting that LTB_4 may have intranuclear functions possibly coupled to lipid homeostasis [116]. In a recent study, it was also reported that 5-LO can interact with several cellular proteins, including coactosine-like protein (CLP) and transforming growth factor type β-receptor-I-associated protein (TRAP-1) [117]. In addition, 5-LO interacts with a third protein which is a human homolog of the protein Dicer, a member of the RNase III family of nucleases, which is implicated in the RNA interference mechanism of gene regulation [117, 118]. However, the biological significance of these protein-protein interactions is presently unclear.

Leukotriene receptors

For LTB_4, two types of surface receptors, with different affinity and cellular expression, are known (BLT_1 and BLT_2). The BLT_1 receptor has been cloned and characterized as a 43-kDa, G-protein-coupled receptor with seven transmembrane-spanning domains (7TM) [119]. The BLT_1 receptor is only expressed in inflammatory cells [120] and shows a high degree of specificity for LTB_4, with a K_d of 0.15 –1 nM [119, 121]. Computer-assisted sequence comparisons revealed that the receptor is distantly related to certain somatostatin receptors as well as some of the chemokine receptors, e.g. those which bind fMLP, LXA_4 and C5a [119, 122].

The role of the BLT_1 receptor has been studied by targeted gene disruption [123, 124]. The receptor was necessary to elicit the physiological effects of LTB_4 (e.g. chemotaxis, calcium mobilization and adhesion to endothelium) and important for the recruitment of leukocytes in an in vivo model of peritonitis. As also observed in mice lacking 5-LO, FLAP or LTA_4 hydrolase, BLT_1 (–/–) mice were protected from the lethal effects of PAF-induced anaphylaxis.

A second G-protein-coupled 7TM receptor for LTB_4, BLT_2, was recently identified [121, 125, 126]. This receptor is homologous to the BLT_1 receptor but has a higher K_d value for LTB_4 (23 nM) and a different ligand specificity and binding profile for various BLT antagonists [127]. In contrast to the BLT_1 receptor, which is predominantly found in leukocytes, BLT_2 is ubiquitously expressed in various tissues.

The genes encoding the LTB_4 receptors are located on chromosome 14 [125, 128]. Interestingly, the open reading frame of the gene encoding the BLT_2 receptor is located within the promoter region of the BLT_1 receptor gene, an unusual gene structure previously not described among mammals [120].

The cys-LTs are recognized by at least two receptor types ($CysLT_1$ and $CysLT_2$), both of which have been cloned and characterized as G-protein-coupled 7TM receptors [129–133]. The $CysLT_1$ receptor contains 336 amino acid residues and messenger RNA (mRNA) is found in, e.g., the spleen, peripheral blood leukocytes, lung tissue, smooth muscle cells and tissue macrophages [130, 132]. The preferred ligands for the $CysLT_1$ receptor are LTD_4 followed by LTC_4 and LTE_4, in decreasing order of potency. The gene encoding the receptor is located on the X chromosome [130].

The $CysLT_2$ receptor contains 345 amino acids with approximately 40% sequence identity to the $CysLT_1$ receptor [129, 131, 133]. This receptor binds LTC_4 and LTD_4 equally well, whereas LTE_4 shows low affinity to the receptor. Studies on the tissue distribution of the $CysLT_2$ receptor show high levels of mRNA in, e.g., heart, brain, peripheral blood leukocytes, spleen, placenta and lymph nodes, whereas only small amounts are found in the lung. The functional role(s) of the $CysLT_2$ receptor is presently unclear, and its wide tissue distribution opens many possibilities, including regulation of brain and cardiac functions.

Acknowledgments. This work was supported by the Swedish Medical Research Council (O3X-10350), the European Union (QLG1-CT-2001-01521), the Vårdal Foundation, the Swedish Foundation for Strategic Research and Konung Gustav V:s 80-Årsfond.

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