Minireview

Intracellular compartmentalization of leukotriene synthesis: unexpected nuclear secrets

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Abstract Leukotrienes are important lipid mediators implicated in the regulation of various cellular processes and in disease states as well as homeostasis. Regulation of leukotriene biosynthesis is therefore of considerable interest. Although the levels of expression and catalytic activity of leukotriene-forming proteins have long been recognized as important determinants of leukotriene biosynthesis, it has recently become apparent that their intracellular compartmentalization also affects the integrated output of this biosynthetic pathway. In this minireview, we focus on the unexpected discovery that the nucleus is the key intracellular site for leukotriene biosynthesis and discuss the mechanisms that regulate protein localization and the potential implications of these findings.

Key words: Leukotriene; 5-Lipoxygenase; Nucleus; Compartmentalization; Metabolon

1. Introduction

Leukotrienes (LTs) are potent mediators of inflammation best known for their role in the pathogenesis of asthma [1]. It is now recognized that they modulate a myriad of cellular processes and participate not only in various inflammatory disease states [2] but also in homeostasis [3]. These lipid mediators are synthesized predominantly by leukocytes via the 5-lipoxygenase (5-LO) pathway of arachidonic acid metabolism (see Fig. 1). In view of the importance of LT actions, substantial effort has been directed at understanding the biochemical, molecular, and cellular aspects of this biosynthetic pathway. As might be expected, each of the key enzymes involved in LT synthesis is subject to regulation at the levels of protein expression and catalytic activity. Only in the last decade has research focused on the intracellular site(s) of LT synthesis. These studies have revealed an unexpected critical role for the cell nucleus as a site at which 5-LO and other LT biosynthetic enzymes are localized. This minireview will focus on the current understanding of the compartmentalization of LT biosynthesis. The localization of 5-LO in resting and activated leukocytes under different physiologic and pathophysiological conditions will be discussed, with emphasis on the molecular mechanisms and catalytic and non-catalytic implications of enzyme compartmentalization.

2. The recognition of a LT biosynthetic metabolon at the nuclear envelope

The first decade of research into LT biosynthesis was dominated by efforts to characterize the relevant enzymes, their cofactors, and their cellular distribution. Little was known about the intracellular sites at which these enzymes functioned. A foundation for our current understanding of such matters derives from studies conducted in the late 1980s and early 1990s, in which crude fractionation methods were used to study the three key proteins involved in the initiation of LT synthesis: cytosolic phospholipase A_2 ($cPLA_2$), 5-LO, and 5-LO activating protein (FLAP). This work demonstrated that, in both resting and activated cells, FLAP was confined to a particulate subcellular fraction, presumably a membrane site [4]. By contrast, $cPLA_2$ [5] and 5-LO [6] were found in the soluble compartment of resting leukocytes, but were redistributed in a Ca^{2+}-dependent fashion to a particulate fraction upon activation.

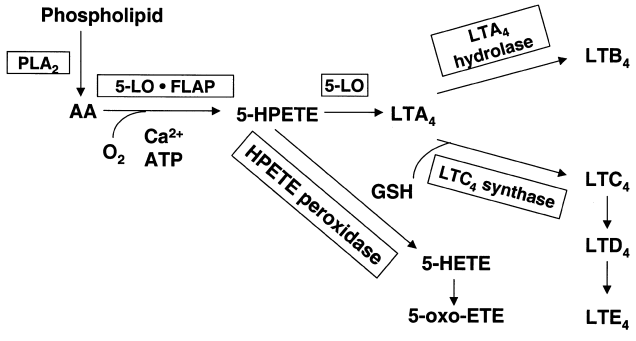

Fig. 1. The 5-LO pathway of arachidonate metabolism leading to the biosynthesis of LTs. HPETE, hydroperoxyeicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid; oxo-ETE, oxo-eicosatetraenoic acid; GSH, reduced glutathione; other abbreviations as defined in the text.

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Abbreviations: LT, leukotriene; 5-LO, 5-lipoxygenase; $cPLA_2$, cytosolic phospholipase A_2; FLAP, 5-LO activating protein

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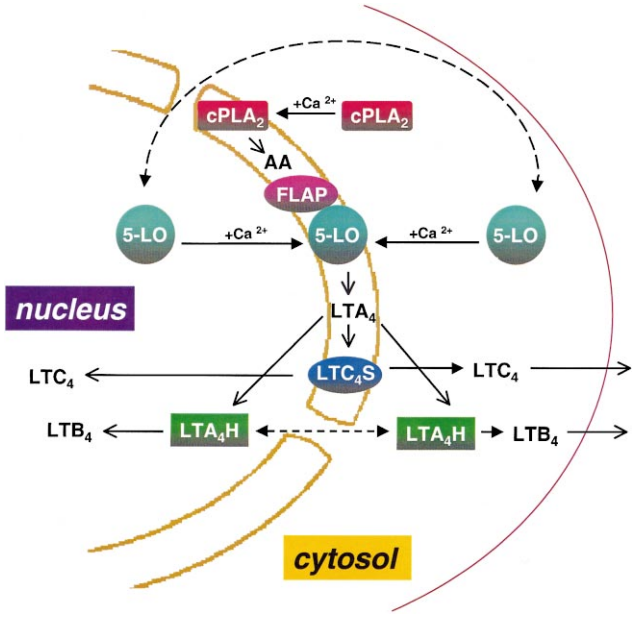

Fig. 2. Intracellular compartmentalization of steps involved in LT biosynthesis. Gaps in the nuclear envelope represent nuclear pores. Solid arrows and filled arrowheads represent translocation of proteins to the nuclear envelope. Dashed lines indicate shuttling of proteins through nuclear pores. Solid lines with unfilled arrowheads represent the generation and flow of metabolic intermediates. Processes depicted between the brown lines are thought to represent those occurring at the inner and/or outer membranes of the nuclear envelope. Abbreviations as defined in the text.

It was inferred from these early studies that all three proteins were co-localized at the same cellular membrane. Moreover, because LTs were recognized to be secreted from leukocytes and to act as paracrine mediators, it was widely assumed that the membrane site at which they were assembled was the plasma membrane. However, none of these assumptions was justified on the basis of the crude methods employed in these initial studies.

In the early to mid-1990s, this issue was definitively addressed by several laboratories in a series of studies that utilized more comprehensive subcellular fractionation methods as well as confocal and ultrastructural immunomicroscopic techniques. Indeed, this body of work demonstrated co-localization of all three proteins in activated leukocytes of various types, including macrophages, monocytes, neutrophils, and mast cells. Surprisingly, however, the site at which these proteins were co-localized was not the plasma membrane, but the nuclear envelope (see Fig. 2). FLAP, an arachidonic acid-binding protein [7], was constitutively localized to the nuclear envelope and perinuclear endoplasmic reticulum [8,9]. $cPLA_2$ was found to translocate from its resting locale in the cytosol to the nuclear envelope upon activation [9–12], and most arachidonic acid released by the actions of $cPLA_2$ was hydrolyzed from nuclear membrane phospholipids [12]. 5-LO was likewise found to translocate to the nuclear envelope upon activation [8,9,13–16]. It was subsequently discovered that LTC_4 synthase, like FLAP, is an integral nuclear envelope protein [17]. Therefore, all the enzymatic steps involved in LTC_4 synthesis were localized to this membrane site.

A macromolecular complex of sequential enzymes in a metabolic pathway has been termed a 'metabolon' [18]. Such a complex can be assumed to result in a cellular microenvironment that allows efficient channeling of metabolic intermediates. There may well be circumstances in which $cPLA_2$ or 5-LO translocate to and function at intracellular sites other than the nuclear envelope, but it would be expected that deviations from the above paradigm would compromise the metabolic efficiency of LT biosynthesis. An important implication of the metabolon concept is that the site of macromolecular assembly is not a random event, but has evolved in a manner that serves the 'wisdom' of the cell. Given that LTs are known to be secreted extracellularly, it is entirely counter-intuitive that their synthesis occurs at the nuclear envelope. This fact strongly suggests that they must subserve important functions within or near the cell nucleus.

3. The complexity of 5-LO compartmentalization in resting leukocytes

Further surprises regarding the localization of 5-LO, in particular, were yet to come. First, the locale of 5-LO in resting leukocytes was found to be cell-specific. The protein was exclusively cytosolic in resting peritoneal macrophages [9], as well as monocytes [13], neutrophils [19], and eosinophils [20,21] isolated from peripheral blood. By contrast, mast cells [15,19] and alveolar macrophages [13,14] contained both cytosolic and intranuclear pools of 5-LO. A constant feature in all cell types was that LT biosynthesis was associated with the translocation of 5-LO to the nuclear envelope. Of course, its site of origin in the cytosol or the nucleoplasm of a resting cell would dictate its eventual association with either the outer or the inner nuclear membrane, respectively, upon activation.

The next surprise was that, even within a given cell type, 5-LO was found to be capable of shuttling in or out of the nucleus in response to in vivo or experimental conditions (Fig. 2). Such movement is unassociated with enzyme activation and LT synthesis, and is to be distinguished from the Ca^{2+}-dependent association with nuclear envelope that accompanies LT synthesis. This shuttling between compartments is presumed to occur via the nuclear pore complex. Examples of dynamic regulation of 5-LO compartmentalization will be discussed later.

4. Molecular mechanisms mediating compartmentalization of 5-LO

Although we have learned a great deal about where 5-LO is localized under different circumstances, our knowledge of the molecular mechanisms which dictate localization is quite rudimentary. It is highly likely that enzyme compartmentalization is determined directly by interactions between specific molecular motifs within the protein and structural components of the cell, or indirectly by interactions with intermediate partner proteins. Candidate motifs within 5-LO that may mediate Ca^{2+}-dependent translocation to the nuclear envelope include putative Ca^{2+}-binding and lipid-binding domains in the N-terminal β-barrel region [22], a src homology-3 binding domain near the C-terminus that may interact with cytoskeletal proteins [23], and one or more of the many consensus sites for phosphorylation by various protein kinases, including tyrosine kinases [24] and mitogen activated protein kinase associated protein kinase [25]. By contrast, shuttling of 5-LO through the nuclear pores of resting cells is almost certainly mediated by motifs functioning as nuclear import and

export sequences that bind to chaperones capable of interacting with the nuclear pore complex. Evidence supports a role for a bipartite nuclear import sequence rich in basic residues near the enzyme's C-terminus [26], and several leucine-rich motifs distributed throughout the protein might serve as candidate nuclear export sequences [27]. It is also likely that the activities of such nuclear import and export sequences are further regulated by phosphorylation reactions.

5. Dynamic regulation of 5-LO compartmentalization

The compartmentalization of 5-LO is subject to dynamic modulation by such factors as culture conditions [20,28], cellular recruitment to tissues [20,28,29], and cytokine exposure [21]. Two examples of this dynamic regulation that have been well studied are discussed for illustrative purposes. The first is observed in the course of tissue-specific macrophage differentiation. Monocytes circulating in peripheral blood are the precursors for all tissue macrophages. They emigrate from the bloodstream, home to various tissues, and differentiate in a tissue-specific fashion into mature tissue macrophages. Monocytes themselves contain predominantly cytosolic 5-LO [13]. Interestingly, this cytosolic distribution is preserved during the course of monocyte differentiation into peritoneal macrophages [9] as well as pulmonary interstitial macrophages [29]. However, the final stage of differentiation into a pulmonary alveolar macrophage, i.e. the process of macrophage emigration through the alveolar wall and into the alveolar space, is uniquely associated with import of 5-LO into the nucleoplasm. This process appears to occur relatively quickly upon cellular entry into the alveolar space, as a prominent intranuclear pool of enzyme is found in virtually all alveolar macrophages, regardless of their duration of residence in the airspace [29]. This distribution of 5-LO in alveolar macrophages, apparently unique among mononuclear phagocytes, may be a consequence of cellular exposure to the unique alveolar milieu. This conclusion is supported by the fact that 5-LO distribution gradually reverts back to the monocyte/non-alveolar macrophage pattern (i.e. predominantly cytosolic pool) during removal of these cells from the alveolar compartment and culture ex vivo over a period of 3 days [29]. This reversal of 5-LO distribution pattern could reflect either export of 5-LO molecules formerly found in the nucleoplasm, or turnover of the intranuclear 5-LO and a failure to import into the nucleus the newly synthesized polypeptide molecules. In preliminary experiments, the nuclear export inhibitor leptomycin failed to prevent this redistribution over time, suggesting a failure of import in cells removed from the alveolus. On the basis of these observations, we suggest that factors unique to the alveolar milieu favor nuclear accumulation of 5-LO, primarily by promoting nuclear import. The precise factors and molecular mechanisms responsible for such import remain to be defined.

A second example of dynamic regulation of 5-LO compartmentalization can be observed in eosinophils. Eosinophils isolated from peripheral blood and studied in suspension contain a predominant cytosolic pool of 5-LO. Upon adherence of these cells on fibronectin, import of 5-LO into the nucleus can be observed over a 30–60 min interval [20]. Such an intranuclear pool of enzyme is also seen in eosinophils that adhere to the vascular wall and emigrate from the bloodstream in the process of recruitment to tissues [20].

6. Effects of 5-LO compartmentalization on LT synthetic capacity

How do these shifts in intracellular distribution influence the capacity for LT synthesis upon subsequent activation? Interestingly, nuclear import of 5-LO increases LT synthetic capacity in fully differentiated alveolar macrophages, but reduces it in adherent eosinophils. These disparate consequences of nuclear import in the two cell types can be attributed, at least in part, to cell-specific differences in the capacities of different enzyme pools to translocate to the nuclear envelope in response to agonist stimulation. Thus, the intranuclear pool of 5-LO in alveolar macrophages translocates readily to the nuclear envelope, while the cytosolic pool does not [14]. In contrast, the intranuclear pool of enzyme in adherent eosinophils translocates far less well upon stimulation than does the cytosolic pool in eosinophils studied in suspension [20]. These results reinforce the critical importance of enzyme translocation for 5-LO activation. A further contrast to both of these situations is provided by the mast cell, in which both cytosolic and intranuclear pools of 5-LO readily translocate to the nuclear envelope upon agonist activation [14]. The reasons for cell- and compartment-specific differences in enzyme translocatability are not known at present, but likely reflect differences in the distribution of critical protein kinases and/or of 5-LO partners which act as anchors or chaperones. It should be acknowledged that contrary results have been obtained with eosinophils under different experimental circumstances. In particular, incubation of eosinophils for 6 h with interleukin-5 has been shown to increase intranuclear 5-LO as well as LT synthetic capacity upon agonist stimulation; however, translocation of enzyme following stimulation was not examined [21].

Another general means by which 5-LO localization may influence the metabolic function of the enzyme involves its capacity to interact with downstream enzymes. The major 5-LO product of alveolar macrophages is LTB_4. Synthesis of this molecule requires the hydrolysis of 5-LO-derived LTA_4 by the enzyme LTA_4 hydrolase. Preliminary data derived from both in situ (immunohistochemical staining of lung tissue) and ex vivo (immunofluorescence microscopy and subcellular fractionation of isolated cells) studies indicate that in alveolar macrophages, LTA_4 hydrolase is found in both cytosolic and intranuclear compartments. In contrast, this enzyme appears to be largely cytosolic in monocytes as well as in structural lung cells such as epithelial cells. These data suggest that nuclear import of the hydrolase accompanies that of 5-LO which occurs during macrophage differentiation in the alveolar space (Fig. 2). The nuclear import of both enzymes in parallel may facilitate efficient coupling of these two sequential reactions at the nucleoplasmic face of the inner nuclear membrane of alveolar macrophages.

Finally, one can envision situations in which compartmentalization of this pivotal enzyme is altered in association with disease states. This could occur on the basis of either inherited or acquired alterations in any of the cellular components that mediate its intracellular distribution, including molecular motifs within 5-LO itself, in signal transduction cascades, or in partner proteins. Increases or decreases in LT biosynthetic capacity could then occur as a result of such alterations, contributing to disease expression.

7. Consequences of the nuclear localization of 5-LO

It is now well accepted that, under most circumstances, translocation of 5-LO to the nuclear envelope is a critical component of enzyme activation. This process allows the enzyme to (1) gain access to substrate arachidonic acid which is hydrolyzed from nuclear membrane phospholipids and presented by FLAP, and (2) couple with downstream enzymes necessary for LT synthesis. The surprising observation that the LT biosynthetic metabolon is found at the nuclear envelope, rather than other membrane sites, surely suggests that LTs or alternative metabolic byproducts such as reactive oxygen species possess important paracrine actions in or near the nucleus. An important goal of future research will be to identify the nuclear targets for the actions of 5-LO products. There is precedent for nuclear eicosanoid receptors [30–32], and the types and roles of such receptors mediating direct intranuclear actions of 5-LO metabolites remain to be elucidated.

In addition, it is becoming increasingly apparent that cells have evolved elaborate mechanisms to be able to dynamically regulate the distribution of 5-LO between cytosol and nucleoplasm of resting cells. There is evidence that this represents one means for modulating the output of the LT biosynthetic pathway. Future studies must elucidate how compartmentalization influences the ability of 5-LO to translocate upon activation and to couple with upstream and downstream components of the metabolon. It is also appealing to speculate that 5-LO might subserve functions beyond its traditional catalytic role. The rationale for this possibility derives from the fact that the enzyme is capable of interacting with other molecules [23,33], and from the observations that even in resting cells not synthesizing LTs, it has been identified in both the euchromatin region of the nucleus (where active gene transcription occurs) [13] and tightly associated with chromatin [19]. The latter findings suggest the intriguing possibility that 5-LO might regulate transcriptional events in the nucleus.

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