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Review

Dynamics of arachidonic acid mobilization by inflammatory cells

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ABSTRACT

The development of mass spectrometry-based techniques is opening new insights into the understanding of arachidonic acid (AA) metabolism. AA incorporation, remodeling and release are collectively controlled by acyltransferases, phospholipases and transacylases that exquisitely regulate the distribution of AA between the different glycerophospholipid species and its mobilization during cellular stimulation. Traditionally, studies involving phospholipid AA metabolism were conducted by using radioactive precursors and scintillation counting from thin layer chromatography separations that provided only information about lipid classes. Today, the input of lipidomic approaches offers the possibility of characterizing and quantifying specific molecular species with great accuracy and within a biological context associated to protein and/or gene expression in a temporal frame. This review summarizes recent results applying mass spectrometry-based lipidomic approaches to the identification of AA-containing glycerophospholipids, phospholipid AA remodeling and synthesis of oxygenated metabolites.

1. Introduction

The traditional view of lipids typically acting as membrane building blocks or fuel has changed dramatically in recent years. Now, it is widely accepted that these biomolecules are centrally involved in cell signaling, and that imbalances in lipid metabolism are causative agents of a number of high-prevalence disorders, ranging from cardiovascular disease, diabetes and obesity to cancer [1–3]. Hundreds to thousands of lipid species can be identified in a given biological system at a cell or tissue levels, each of them with a particular distribution and function [4].

The significant advances in analytical techniques over the last years, especially mass spectrometry (MS) and the parallel improvement in molecular ionization, together with the possibility to use this technique as a detector in chromatographic separation, have constituted a breakthrough in lipid analysis and, therefore, a better understanding of lipid biochemistry has been possible. This has contributed to the development of lipidomics [5–7], a branch of metabolomics that pursues a thorough scientific study of lipids. Lipidomics not only aims at characterizing and analyzing lipid species in a particular state of the cell — called the *static* composition — but also aims at integrating the understanding of lipid pathways (synthesis, remodeling or transport) with their biological roles and with gene regulation and protein expression [5,7–11].

There are two major strategies for lipidomic analysis. The first one, called global lipidomics, pursues the analysis of the whole lipidome of biological samples, by using either direct infusion methods or previous liquid chromatography steps. On the other hand, targeted or focused lipidomics, is based on setting conditions for analysis of specific categories of lipids, thus improving sensitivity [11–14].

As lipidomics is focused on profiling the *static* composition of molecular species in biological samples, the term metabolipidomics has been coined to emphasize the dynamical aspects of lipids in cells, organs, tissues and whole organisms [15]. In this case, lipids containing stable isotopes are used to follow their metabolic fate through the various possible pathways, thus facilitating the identification of minor species with rapid turnover rates.

2. Cellular utilization of arachidonic acid

Arachidonic acid (5,8,11,14-eicosatetraenoic acid) is an ω-6 essential fatty acid, obtained directly from diet or, alternatively, synthesized from linoleic acid (18:2n−6) through the successive actions of Δ6-desaturase, elongase and Δ5-desaturase, which occurs mainly in liver but also in other tissues (Fig. 1). In western diets it is calculated that the intake of AA is 0.2–0.3 g/day, whereas the intake of linoleic acid is 10–20 g/day, indicating that the amount of AA coming from linoleic acid is higher than that coming directly from the diet [16,17].

AA is the common precursor of the eicosanoids, a family of lipid mediators with key roles in physiology and pathophysiology, particularly

Abbreviations: AA, arachidonic acid; PLA_2, phospholipase A_2; $cPLA_2\alpha$, group IVA cytosolic PLA_2; $iPLA_2$, Ca^{2+}-independent PLA_2; $sPLA_2$, secreted PLA_2; CoA-IT, CoA-independent transacylase; ACS, acyl-CoA synthetase; ESI, electrospray ionization; HPLC, high performance liquid chromatography; MS, mass spectrometry; TLC, thin layer chromatography; PC, choline glycerophospholipids; PE, ethanolamine glycerophospholipids; PI, phosphatidylinositol

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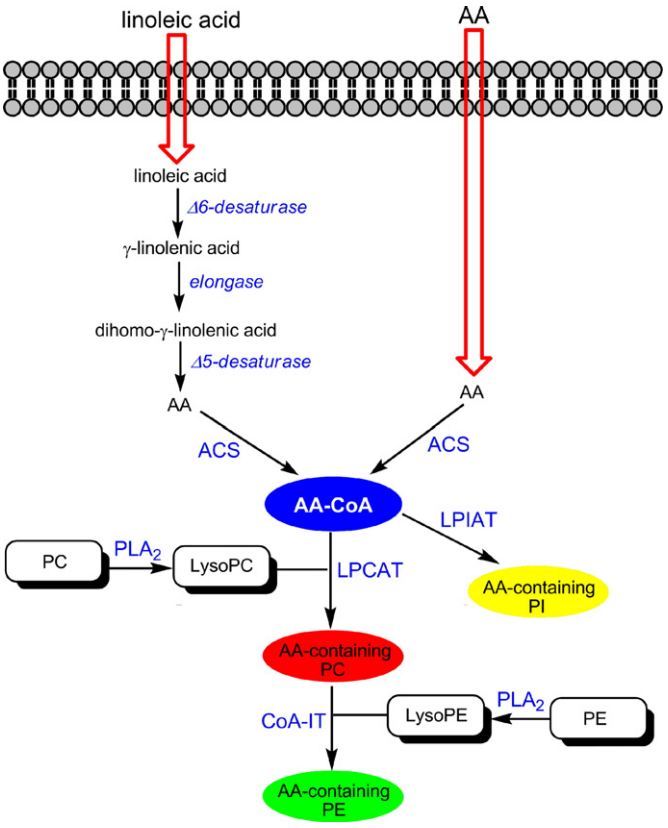

Fig. 1. AA incorporation in glycerophospholipids. AA is obtained directly from diet or synthesized from linoleic acid. Afterward, AA is incorporated into the *sn*-2 position of glycerophospholipids and later subjected to remodeling processes via CoA-IT-dependent reactions. ACS: acyl:CoA synthetase; LPCAT: lysophosphatidylcholine:acyl-CoA acyltransferase; PLA_2: phospholipase A_2; CoA-IT: independent-CoA transacylase.

in inflammatory reactions [18,19], although the fatty acid can exert potent biological roles by itself, e.g. by inducing apoptosis [20–22]. Additionally, when present in elevated concentrations, AA can give rise to significant quantities of its 2-carbon elongation product, adrenic acid (22:4n−6). Adrenic acid is the precursor of 22-carbon 1*a*,1*b*-dihomologue prostaglandins (dihomoprostaglandins) [23–26]. The potent biological activity of the eicosanoids compels the cells to tightly control the levels of free AA in such a manner that availability of free AA is frequently a rate-limiting step in eicosanoid generation [27,28].

In inflammatory cells, AA is generally found esterified in the *sn*-2 position of glycerophospholipids, particularly choline glycerophospholipids (PC), ethanolamine glycerophospholipids (PE), and phosphatidylinositol (PI) [28]. Cellular free AA levels are controlled by two competing reactions; on one hand, the phospholipase A_2 (PLA_2)-mediated cleavage of the *sn*-2 position of phospholipids to yield the free fatty acid and, on the other, the CoA-dependent acyltransferase-modulated acylation reactions that re-incorporate the free fatty acid into phospholipids [29,30]. In resting unstimulated cells, the reacylation reactions dominate over the PLA_2-mediated deacylation step; thus free AA is kept at very low levels. Stimulation of the cells by receptor agonists results in the activation of intracellular PLA_2s [31–35]. Under these conditions, the rate of AA release clearly exceeds that of reincorporation into phospholipids; hence, net accumulation of free AA occurs that is followed by its conversion into various eicosanoids. Nevertheless, AA reacylation reactions are still very significant under stimulated conditions, as manifested by the fact that only a minor fraction of the free AA released by PLA_2s is converted into eicosanoids, the remainder being effectively incorporated back into phospholipids [36,37].

The first step for AA incorporation into cellular lipids is the activation of the carboxyl group of the fatty acid by thioesterification with CoA. This reaction is catalyzed by acyl-CoA synthetases, of which there are many in cells [30]. In the context of AA metabolism it is worth mentioning the long chain acyl-CoA synthetases ACSL3, 4 and 6, as they show some selectivity toward AA [38–40]. The next step in the AA incorporation is the esterification of the fatty acid into primarily the *sn*-2 position of glycerophospholipids, a reaction that is carried out by lysophospholipid:acyl CoA acyltransferases, in particular those of the membrane-bound O-acyltransferase family (MBOAT) [41]. Those that have been found to exhibit clear preference for AA include lysoPC:acyl-CoA acyltransferase 2 [42], lysoPC:acyl-CoA acyltransferase 3 [43–45], lysoPI:acyl-CoA acyltransferase and lysophosphatidic acid:acyl-CoA acyltransferase 3 [44,46,47]. In addition, the lipase CGI-58 shows lysophosphatidic:acyl-CoA acyltransferase with high preference toward AA [48]. For detailed information on the biochemistry and functioning of CoA-dependent acyl transferases, the reader is kindly referred to recent reviews on the subject [30,49–51].

AA bound to phospholipid is also the subject of successive transacylation reactions aimed at ensuring the proper distribution of the fatty acid within the various cellular phospholipid pools [29,30,52–54]. This appears to be important not only for membrane homeostasis but also for the execution of appropriate cell responses during physiological and pathophysiological activation [55–59]. These transacylation reactions are catalyzed by CoA-independent transacylase (CoA-IT), an enzyme that transfers AA moieties preferentially from diacyl PC species to PE plasmalogens [29,30] (Fig. 1). The sequence of CoA-IT is yet to be described. Thus, currently the only manner to study the cell regulation of CoA-IT is by following its enzyme activity [60,61].

Once the AA has been effectively incorporated into phospholipids the fatty acid can be eventually liberated, especially under cell stimulation conditions, by a number of PLA_2 enzymes. So far, more than 30 enzymes possessing PLA_2 activity have been described in mammals [62–64]. Attending to sequence homology criteria, the PLA_2s have been classified into 16 groups [62–65], although based on biochemical features they can be grouped into 5 main families, namely the Ca^{2+}-dependent secreted PLA_2s (sPLA_2), Ca^{2+}-dependent cytosolic PLA_2s (cPLA_2), Ca^{2+}-independent cytosolic PLA_2s (iPLA_2), platelet-activating factor acetylhydrolases, and lysosomal PLA_2s. Of these, the first two have been repeatedly implicated in receptor-mediated AA mobilization in response to a variety of stimuli. Today, it is firmly established that the calcium-dependent cytosolic group IVA $PLA_2\alpha$ (c$PLA_2\alpha$) is the critical enzyme in stimulus-dependent AA mobilization and that, depending on cell type and stimulation conditions, a sPLA_2 may also participate by amplifying the c$PLA_2\alpha$-regulated response [66–91]. Under some conditions, the Ca^{2+}-independent group VIA iPLA_2 may also effect the AA release [92–94] but in other conditions, it mediates phospholipid reacylation reactions by regulating the steady-state level of lysoPC [95–97], reflecting the multiplicity of functions that this enzyme appears to serve depending on cell type [34,98]. For detailed information on the cellular regulation of AA mobilization by PLA_2 enzymes, the reader is kindly referred to the many comprehensive reviews that have been published in recent years covering different aspects of the subject [31–35,51,62–65,99–104].

After the AA has been released from phospholipids under stimulation conditions, it can be metabolized into eicosanoids through four different pathways, namely cyclooxygenase, lipoxygenase, cytochrome-P450 and oxygen species-triggered reactions (Fig. 2). These pathways yield a plethora of compounds, such as prostaglandins, isoprostanes, thromboxane, leukotrienes, lipoxins and epoxyeicosatrienoic acids, all of which act in a paracrine/autocrine manner through specific receptors on the plasma membrane.

For the biosynthesis of prostaglandins and thromboxane from AA, synthesis of PGH_2 via cyclooxygenase is the first step. Cyclooxygenase incorporates molecular O_2 and forms PGG_2 that is subsequently reduced to form PGH_2 by the action of the peroxidase active site. PGH_2 is the substrate of different prostaglandin and thromboxane synthases that are expressed in tissues and cells in a selective-manner and lead to the formation of PGE_2, PGD_2, PGI_2, $PGF_2\alpha$ and TXA_2 [105]. These compounds