Plasmalogens, Arachidonic Acid, and Phospholipase A₂

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Plasmalogens are an important family of glycerophospholipids which characteristically contain a monounsaturated fatty alcohol linked to the sn-1 carbon of glycerol via a vinyl ether linkage (i.e. the double bond of the fatty alcohol is conjugated with the ether oxygen). There are two major types of plasmalogens in mammalian cells, those containing either choline or ethanolamine as the headgroup. While heart contains relatively high amounts of choline plasmalogens, ethanolamine plasmalogens are particularly abundant in innate immune cells such as monocytes and macrophages. Notably, in the latter cells, practically only polyunsaturated fatty acids of both the omega-6 and omega-3 series are found at the sn-2 position of ethanolamine plasmalogens, with arachidonic acid being the most abundant. Owing to such fatty acid composition, the importance of this class of lipids to phosphlipase A₂-regulated pathways leading to generation of lipid mediators has long been recognized.

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Plasmalogens received this name after the finding by Feilgen and Voigt in 1924 that their vinyl ether bond breaks to form an aldehyde in the presence of mercuric chloride. The aldehyde reacted with a fuchsine-sulfurous acid stain, coloring purple-bluish the cytoplasm of cells but not the nucleus. Hence, the molecules generating the aldehyde were named plasmalogens because they colored the cellular "plasmal", or cell's interior. From then on, this reaction came to be universally known as the "plasmal reaction."

Mammalian cells contain significant amounts of a relatively lesser known enzyme that shows marked specificity for ether lipids containing arachidonic acid (AA) and other 20–22 carbon fatty acids. This enzyme is called CoA-independent transacylase (CoA-IT), and transfers polyunsaturated moieties of 20–22 carbons, typically AA, from diacyl PC species to ether phospholipids, particularly the ethanolamine plasmalogens. It is important to note however, that CoA-IT may use 1-acyl-PE just as well as 1-alkenyl-PE. The CoA-catalyzed reaction is considered of special relevance not only for the maintenance of membrane homeostasis but also for ensuring the appropriate distribution of AA among the various phospholipid pools for the execution of phospholipase A₂-dependent responses during immune cell activation. Thus, CoA-IT is regarded as a key enzyme for the regulation of polyunsaturated fatty acid mobilization reactions in major immunoinflammatory cells [1].

CoA-IT catalyzes the enzymatic step that is unique to phospholipid AA remodeling pathways, i.e. the direct transfer of AA from a phospholipid donor to a lysophospholipid acceptor in the absence of CoA or ATP. Although the gene sequence of CoA-IT has not been identified so far, its activity has been relatively well characterized in broken cell preparations, and pharmacological inhibitors have been identified. Moreover, the enzyme can also be followed in whole cells by determining the pattern of radiolabeling of PC versus PE over time, as done in the present study. Results from previous studies have defined CoA-IT as a membrane-bound, calcium-independent
enzyme. Based on biochemical and mechanistic commonalities, it was suggested that CoA-independent transacylation reactions in cells are catalyzed by (an) enzyme(s) of the phospholipase A2 (PLA2) family, and the suggestion was made that group IVC cytosolic phospholipase A2 (also called cytosolic phospholipase A2γ, cPLA2γ) could constitute a prime candidate [2].

By using siRNA technology to specifically knock down cellular expression levels of cPLA2γ, we have tested experimentally the proposed involvement of cPLA2γ in regulating phospholipid AA remodeling. Our results clearly indicate that this is the case, as cells which were made deficient in cPLA2γ, transfer AA from PC to PE significantly more slowly than control cells. Thus the finding is significant because it makes now possible to apply molecular biology approaches, such as overexpression or deletion, which could significantly expand our knowledge about the cellular and molecular regulation of phospholipid AA remodeling reactions [2].

**Slide 1.** Representative structures of 1-acyl, 1-alkyl, or 1-alkenyl glycerophospholipids. 1-Alkenyl glycerophospholipids are called plasmalogens and annotated with a “p” before the sn-1 hydrocarbon chain abbreviation.

**Slide 2.** The sn-2 fatty acid composition of major plasmalogens isolated from peritoneal macrophages (p16:0, p18:1 and p18:0) reveals a marked enrichment with AA (20:4).

**Slide 3.** Characterization of plasmalogen-deficient RAW.12 cells. Despite the considerable absence of ether phospholipids in RAW.12 cells (including ethanolamine plasmalogens and alkyl-PC species), their AA content was found to be the same as that of native RAW264.7 cells, as quantified by gas chromatography/mass spectrometry. The distribution of AA between phospholipid classes was also preserved in the RAW.12 cell compared to native RAW264.7.

**Slide 4.** AA-containing phospholipid species in RAW 264.7 cells and plasmalogen-deficient variants. The profile of AA-containing PC, PS, PE, PI species was determined for RAW 264.7 cells and plasmalogen-deficient variants by LC/MS. There is a compensatory elevation of the levels of AA in diacyl species in the plasmalogen-deficient cells.

**Slide 5.** Phospholipid AA remodeling in RAW 264.7 cells and plasmalogen-deficient variants. RAW 264.7 cells were pulse-labeled with [3H]AA, washed and incubated without label for the indicated periods of time. Phospholipids were separated into classes by thin-layer chromatography. The radioactivity incorporated into each phospholipid class was determined by scintillation counting, and the remodeling time (time at which the radioactivity content of PC equals that of PE) was determined.

**Slide 6.** Phospholipase A2 role in phospholipid AA remodeling. RAW 264.7 cells were pulse-labeled with [3H]AA, washed and incubated without label for the indicated periods of time in the absence (none) or presence of the following inhibitors: 2 µM pyrrophenone, 10 µM GK241, 5 µM bromoenol lactone, 10 µM FKGK18, 5 µM GK436. The radioactivity incorporated into each phospholipid class was determined by scintillation counting, and the remodeling time (time at which the radioactivity content of PC equals that of PE) was determined. Phospholipid AA remodeling was also analyzed in cells treated with a scrambled siRNA (control) or siRNA targeting cPLA2γ.

**Slide 7.** Stimulated AA mobilization in RAW 264.7 cells. [3H]AA-labeled cells, treated with a scrambled siRNA (control) or siRNA for cPLA2γ, were left untreated or treated with 150 µg/ml zymosan for 1 h in the absence or presence of a number of PLA2 inhibitors: 2 µM pyrrophenone, 10 µM GK241, 5 µM bromoenol lactone, 10 µM FKGK18, 5 µM GK436.
REFERENCES


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