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Serial Review: Redox-Regulated Phospholipase Signal Transduction
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Role of cytochrome P450 in phospholipase A2- and arachidonic acid-mediated cytotoxicity☆

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Abstract

Phospholipases A2 (PLA2) comprise a set of extracellular and intracellular enzymes that catalyze the hydrolysis of the sn-2 fatty acyl bond of phospholipids to yield fatty acids and lysophospholipids. The PLA2 reaction is the primary pathway through which arachidonic acid (AA) is released from phospholipids. PLA2s have an important role in cellular death that occurs via necrosis or apoptosis. Several reports support the hypothesis that unesterified arachidonic acid in cells is a signal for the induction of apoptosis. However, most of the biological effects of arachidonic acid are attributable to its metabolism by mainly three different groups of enzymes: cytochromes P450, cyclooxygenases, and lipoxygenases. In this review we will focus on the role of cytochrome P450 in AA metabolism and toxicity. The major pathways of arachidonic acid metabolism catalyzed by cytochrome P450 generate metabolites that are subdivided into two groups: the epoxyeicosatrienoic acids, formed by CYP epoxygenases, and the arachidonic acid derivatives that are hydroxylated at or near the ω-terminus by CYP ω-oxidases. In addition, autoxidation of AA by cytochrome P450-derived reactive oxygen species produces lipid hydroperoxides as primary oxidation products. In some cellular models of toxicity, cytochrome P450 activity exacerbates PLA2- and AA-dependent injury, mainly through the production of oxygen radicals that promote lipid peroxidation or production of metabolites that alter Ca^{2+} homeostasis. In contrast, in other situations, cytochrome P450 metabolism of AA is protective, mainly by lowering levels of unesterified AA and by production of metabolites that activate antiapoptotic pathways. Several lines of evidence point to the combined action of phospholipase A2 and cytochrome P450 as central in the mechanism of cellular injury in several human diseases, such as alcoholic liver disease and myocardial reperfusion injury. Inhibition of specific PLA2 and cytochrome P450 isoforms may represent novel therapeutic strategies against these diseases.

Keywords: Phospholipase A2; Cytochrome P450; Arachidonic acid; Oxidative stress; Epoxyeicosatrienoic acids; Hydroxyeicosatetraenoic acids; Lipid hydroperoxides; Lipid peroxidation; Free radicals

Contents

Phospholipase A2 classification . . . 365
Role of phospholipase A2 in cellular injury . . . 365
Metabolism of arachidonic acid by cytochrome P450 . . . 367
Role of cytochrome P450 in PLA2- and AA-mediated cytotoxicity . . . 368
Cytotoxicity produced by arachidonic acid in liver cells overexpressing CYP2E1 . . . 368
Role of phospholipase A2 activation and calcium in CYP2E1-dependent toxicity in HepG2 cells . . . 369

Abbreviations: PLA2, phospholipase A2; AA, arachidonic acid; EET, epoxyeicosatrienoic acid; sPLA2, secreted phospholipase A2; cPLA2, calcium-dependent phospholipase A2; iPLA2, calcium-independent phospholipase A2; HETE, hydroxyeicosatetraenoic acid; ROS, reactive oxygen species; COX, cyclooxygenase; LOX, lipoxygenase; CIF, calcium influx factor; PCT, porphyria cutanea tarda; HCB, hexachlorobenzene.

☆ This article is part of a series of reviews on "Redox-Regulated Phospholipase Signal Transduction." The full list of papers may be found on the home page of the journal.

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Regulation of AA-mediated apoptosis by cytochrome P450 . . . 370
Role of cytochrome P450 and capacitative calcium entry in cell death . . . 370
Role of cytochrome P450 and phospholipase A2 in the toxicity of xenobiotics . . . 370
Role of phospholipase A2 and cytochrome P450 in cell injury in human diseases . . . 371
Alcoholic liver disease . . . 371
Myocardial reperfusion injury . . . 371
Porphyria cutanea tarda (PCT) . . . 371
Cerebral ischemia . . . 371
Summary . . . 372
Future directions . . . 372
References . . . 372

Phospholipase A2 classification

Phospholipases A2 (PLA2) comprise a set of extracellular and intracellular enzymes that catalyze the hydrolysis of the sn-2 fatty acyl bond of phospholipids to yield fatty acids and lysophospholipids [1]. The extracellular (secreted) PLA2s (sPLA2) have low molecular masses (13–18 kDa), require millimolar calcium concentrations for catalytic activity, and do not manifest significant fatty acid selectivity in vitro. In mammalian cells, as many as six different sPLA2 groups exist: groups IB, IIA, IIC–F, III, V, X, and XII. The intracellular PLA2s are further divided into cPLA2 (cytosolic calcium dependent, group IV) and iPLA2 (cytosolic calcium independent, group VI), based on the Ca^{2+} requirements needed for basal activity. cPLA2 requires micromolar Ca^{2+} for membrane translocation but not for catalysis, possesses a preference for phospholipids containing AA, and have high molecular mass (>60 kDa). Ceramide 1-phosphate binding [2] and PKC and ERK1/2-mediated phosphorylation of cPLA2 modulate membrane binding and catalytic activity. iPLA2 exhibits no substrate specificity for AA-containing phospholipids and no Ca^{2+} requirement for activity and has high molecular mass (about 85 kDa) [1,3,4]. There is also a class of PLA2s called platelet-activating factor (PAF) acetylhydrolases, whose primary substrate is PAF, from which they hydrolyze an acetyl moiety present at the sn-2 position, which will not be discussed further here [5].

Role of phospholipase A2 in cellular injury

The PLA2 reaction is the primary pathway through which AA is released from phospholipids [1]. Phospholipase A2 activation results in the degradation of membrane phospholipids and accumulation of unsaturated free fatty acids and lysophospholipids. Lysophospholipids may perturb membrane homeostasis and increase membrane fluidity and permeability [6]. Arachidonic acid can directly affect ion channels [7,8], increase the mitochondrial permeability transition pore open time [9], and activate ERK, JNK, and p38 MAPKs [10]. However, most of the biological effects of arachidonic acid are attributable to its metabolism by mainly three different groups of enzymes: cyclooxygenases, lipoxygenases, and cytochromes P450. Metabolism of free arachidonic acid by cyclooxygenases leads to the formation of prostaglandins, prostacyclin, and thromboxanes, with important roles in numerous physiological and pathophysiological processes such as vasodilation and vasoconstriction, inflammation, thrombosis, ovulation, mitogenesis, renal function, etc. There are two cyclooxygenase isoenzymes: (i) COX-1, molecular weight 67 kDa, ubiquitously and constitutively expressed in mammalian tissues and cells, localized in the endoplasmic reticulum; and (ii) COX-2, molecular weight 72 kDa, generally present in mammalian tissues at very low levels, highly inducible by many types of stimuli such as cytokines and growth factors, localized in the endoplasmic reticulum and nuclear envelope [11]. Metabolism of free arachidonic acid by 5-lipoxygenase leads to the formation of leukotrienes, with important functions as mediators of a variety of inflammatory and allergic reactions. In contrast to prostaglandins, leukotrienes are made predominantly by inflammatory cells like polymorphonuclear leukocytes, macrophages, and mast cells [11]. 5-Lipoxygenase is a 78-kDa, Ca^{2+}- and ATP-dependent enzyme that, once activated, translocates from the cytosol to either the nuclear or the plasma membrane compartment [12].

Metabolism of free arachidonic acid by cytochrome P450 leads to the formation of AA epoxygenase products, AA ω/ω-1 hydroxylase products, lipoxygenase-like products, and free radical oxidation products (see below and Fig. 1) [13,14]. The cytochrome P450 enzymes comprise a large superfamily of proteins, abbreviated as CYP enzymes, classified in different families (denoted by an Arabic numeral) and subfamilies (indicated by a letter) in accordance with the degree of homology of amino acid sequence in their protein structure. In mammals, 14 families and 26 subfamilies of cytochromes P450 have been identified. Cytochromes P450 can be found in nearly every tissue, being more abundantly expressed in the liver, and are localized in the endoplasmic reticulum and mitochondria (molecular weight 50–60 kDa) [15]. In this review we will focus on the role of cytochrome P450 in AA metabolism and toxicity.

PLA2s have an important role in cellular death that occurs via necrosis or apoptosis. In necrosis, cell death is characterized by cell and organelle swelling, ATP depletion, and increased plasma membrane permeability and macromolecule release. It has been proposed that during necrosis, PLA2 activity increases, producing accelerated membrane phospholipid hydrolysis and, in turn, increased plasma membrane permeability and cell lysis [16]. In support of this hypothesis, LDH