

Phospholipase A₂

Key References

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Overview

Phospholipase A₂ (PLA₂) designates a class of enzymes that hydrolyze the sn-2 ester of glycerophospholipids to produce a fatty acid and a lysophospholipid. It has become clear that some of these enzymes liberate arachidonic acid in mammalian cells for the biosynthesis of eicosanoids, and thus there has been considerable interest in developing PLA₂ inhibitors. Based on amino acid sequences, there are now more than 12 distinct groups of mammalian PLA₂s, as well as many non-mammalian forms, all of which have been classified into 14 distinct groups with many subgroups. The mammalian enzymes are listed in the accompanying Table, together with some of their properties and information on inhibitors.

Since naturally occurring phospholipids have virtually no solubility in water, PLA₂ must bind to the lipid-water interface to access its substrate, and it is clear that catalysis occurs at the interface (interfacial catalysis). One has to be careful with the interpretation of kinetic data including inhibition data, and in fact many of the previously reported PLA₂ inhibitors work by non-specific mechanisms. One of the problems is that the substrate forms an interface, and the inhibitor can potentially partition into the membrane phase. Thus, inhibition of a PLA₂ by micromolar amounts of a compound using micromolar amounts of substrate may occur because a large fraction of the interface could be occupied by inhibitor. This may change the physical nature of the interface, causing the enzyme to desorb from the membrane into the aqueous phase, resulting in non-specific inhibition. Inhibitors that work by this non-specific mechanism are clearly not useful for studying the role of PLA₂s in complex cellular pro-

cesses. Among those inhibitors that bind tightly to the active site of PLA₂s, and thus operate by a specific mechanism, the issue of PLA₂ group specificity is important, and this issue is addressed in the Table below.

To date, 10 groups of mammalian secreted PLA₂s, known as s-PLA₂s, have been identified. Group IB PLA₂, also known as pancreatic PLA₂, is found not only as part of the digestive fluid where it functions to hydrolyze dietary phospholipids, but also in non-digestive tissues including spleen where it has unknown functions. Group IIA PLA₂ was the first non-pancreatic mammalian PLA₂ to be identified as a component of synovial fluid and platelets. This enzyme is pro-inflammatory, displays potent bacterial properties and is a target for the development of anti-inflammatory agents including anti-sepsis agents. Groups IIC, IID, IIE, IIF, III, X, XIIA and XIIB secreted PLA₂ were discovered by recombinant DNA techniques. The group IIC gene is functional in mice but occurs as a pseudogene in humans. Group XIIB is best designated as a secreted PLA₂-like protein since it has a natural mutation of a key catalytic residue that renders this protein devoid of phospholipase activity. The function of these recently discovered secreted PLA₂s are unknown. Group V PLA₂, also discovered at the DNA level, is an active enzyme secreted from macrophages and probably a variety of other cells. Recent gene disruption studies implicate a role of this enzyme in arachidonic acid release in stimulated macrophages.

All of these secreted PLA₂s have similar size, three-dimensional structure, and active site residues (except for group XIIB as noted above). They require submillimolar amounts of calcium for catalytic

activity. Potent inhibitors are known for some of these enzymes as shown in the Table

Mammalian cells also contain two intracellular enzymes that act on long-chain phospholipids. Group IVA PLA₂ translocates from the cytosol to internal membranes in response to micromolar calcium, and shows specificity for arachidonyl-containing phospholipids. A wide variety of studies have shown that this enzyme, also known as cPLA₂α, releases arachidonic acid from membrane phospholipids for the biosynthesis of eicosanoids. Recent paralogs of cPLA₂α have been identified in the genome, but their functions are not known. It has been proposed that the calcium-independent group VI PLA₂ may be involved in phospholipid remodeling, insulin secretion from β cells and in store-operated calcium entry. Groups VII and VIII PLA₂s are highly specific for phospholipids with short sn-2 chains and are thought to terminate the action of platelet activation factor by hydrolyzing the sn-2 ester and to act on oxidized phospholipids.

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GROUP	IB (P8913, P6534, P0861)	IIA, IIC, IID, IIE and IIF	III	IV	V
SOURCES ^b	Mammals	Mammals, IIC gene is functional in mice but a pseudogene in humans	Mammals, bee and wasp venoms	Mammals	Mammals
SECRETED OR CYTOSOLIC	Secreted	Secreted	Secreted	Cytosolic	Secreted
STRUCTURAL INFORMATION	133 aa (human), multiple disulfides	~130 aa, multiple disulfides, IIF has a C-terminal extension	Mammalian enzyme has a ~16 kDa group III PLA ₂ domain and long N- and C-terminal extensions	749 aa (human) α-paralog, several new paralogs have been recently identified	118 aa (human), multiple disulfides
MOLECULAR WEIGHT (kDa)	13 - 15	13 - 17	55 (mammalian), 18 (venom)	80-85 (α-paralog)	14
COFACTOR	μM – mM Ca ²⁺ required for active site substrate binding and catalysis ^e	μM – mM Ca ²⁺ required for active site substrate binding and catalysis ^e	μM – mM Ca ²⁺ required for active site substrate binding and catalysis ^e	sub to low μM Ca ²⁺ required for membrane binding, γ-paralog does not require Ca ²⁺	μM – mM Ca ²⁺ required for active site substrate binding and catalysis ^e
TIGHT BINDING INHIBITORS ^c	HK series sn-2-amide phospholipids, Indole analogs ^f	HK series sn-2-amide phospholipids, indole analogs ^e	HK series sn-2-amide phospholipids, indole analogs ^e	HK series sn-2-amide phospholipids, indole analogs ^f	HK series sn-2-amide phospholipids, indole analogs ^e
CONTROL COMPOUNDS	Enantiomer of HK series, some indole analogs	Enantiomer of HK series, some indole analogs	Enantiomer of HK series, some indole analogs	AACOCH3	Enantiomer of HK series, some indole analogs
INHIBITOR SPECIFICITY	Many group IB inhibitors inhibit other secreted PLA ₂ s ^e	Many group II inhibitors inhibit other secreted PLA ₂ s ^e	Many group III inhibitors inhibit other secreted PLA ₂ s ^e	AACOFC3 and MAFP also inhibit group VI PLA ₂ s	Many group V inhibitors inhibit other secreted PLA ₂ s ^e
TISSUE EXPRESSION ^b	Pancreatic juice, spleen, lung	Several mammalian tissues, tissue expression is partially overlapping	Kidney, heart, liver and skeletal muscle	Most mammalian tissues	Macrophages, lung
PHYSIOLOGICAL FUNCTION ^d	Digestion of dietary phospholipids	Bactericidal activity (IIA), functions of IIC, IID, IIE and IIF are unknown	Not known	Agonist-triggered arachidonic acid release for eicosanoid and platelet activator factor production (α-isoform), functions of other isoforms are unknown	Contributes to arachidonic acid release for eicosanoid production, augmentation of cPLA ₂ α function
DISEASE RELEVANCE ^d	Mouse IB deficient mice show resistance to obesity	Sepsis and inflammation (possibly arthritis) (IIA)	Not known	Inflammation and allergy related to lipid mediator production	Not known

FOOTNOTES

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GROUP	VI	VII	VIII	X	XIIA
SOURCES ^b	Mammals	Human plasma	Bovine brain	Mammals	Mammals
SECRETED OR CYTOSOLIC	Cytosolic	Secreted	Cytosolic	Secreted	Secreted
STRUCTURAL INFORMATION	iPLA2a isoform is 752 aa (human), several isoforms and splice forms are known	420 aa (Human)	230 aa (bovine γ -subunit)	123 aa (human)	167 aa (human), multiple disulfides
MOLECULAR WEIGHT (kDa)	iPLA2a isoform 80 - 85	45	29	14	19
COFACTOR	None, may be augmented by ATP	Not known	Not known	μM – mM Ca ²⁺ required for active site substrate binding and catalysis ^e	μM – mM Ca ²⁺ required for active site substrate binding and catalysis ^e
TIGHT BINDING INHIBITORS ^c	BEL (B1552) AACOCF ₃ (A231) MAFP	Patented inhibitors from GSK	Not known	AACOCF ₃ (A231), MAFP, pyrrophenone and related compounds	Not known
CONTROL COMPOUNDS	Enantiomers BEL (B1552) have distinct specificity for the various iPLA2 isoforms	Not known	Not known	Enantiomer of HK series, some indole analogs	Not known
INHIBITOR SPECIFICITY	AACOCF ₃ and MAFP also inhibit cPLA _{2α}	Not known	Not known	Many group IB inhibitors inhibit other secreted PLA ₂ s ^e	Not known
TISSUE EXPRESSION ^b	Expressed in many mammalian tissues	Human plasma, an isoform is expressed in mammalian cytosol	Brain	Several mammalian tissues	Several mammalian tissues
PHYSIOLOGICAL FUNCTION ^d	Stores-operated calcium entry, insulin secretion, phospholipid acyl chain remodeling (although still controversial)	Serum form degrades platelet activating factor and phospholipids with oxidized fatty acyl chains	Not known	Not known	Not known
DISEASE RELEVANCE ^d	Not known	Not known	Mutations cause Miller-Dieker lissencephaly	Not known	Not known

Abbreviations

AACH(OH)CF₃: 2-Hydroxy-1,1,1-trifluoro-6,9,12,15-heneicosatetraene

AACOCF₃: 2-oxo-1,1,1-Trifluoro-6,9-12,15-heneicosatetraene

AACOCH₃: 2-oxo-6,9,12,15-Heneicosatetraene

BEL: (E)-6-(Bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyran-2-one

MAFP: 4,7,10,13-Nonadecatetraenyl fluorophosphonic acid methyl ester

SB-222657: N-[6-(4-Chlorophenyl)hexyl]-2-oxo-4-[(S)-(phenylmethyl)sulfinyl]-1-azetidineacetamide

SB-223777: N-[6-(4-Chlorophenyl)hexyl]-2-oxo-4-[(R)-(phenylmethyl)sulfinyl]-1-azetidineacetamide

FOOTNOTES

a Not included in the table are a variety of other PLA₂s from non-mammalian sources including PLA₂s in snake and insect venoms, for example the group IA enzymes in cobra and krait venoms (**P4034**, **P7778**, **P6139**), rattlesnakes (P377) and bee venom (**P9279**).

b The species and tissue distribution is only a partial listing.

c Only those inhibitors that have been shown to bind specifically to the active site of the PLA₂s have been listed (see text for more discussion), and the list is not necessarily comprehensive.

d The list of physiological functions and disease relevance is only partial as the functions of PLA₂s are under active investigation and are still unresolved in many cases.

e See, Singer et al., *J. Biol. Chem.*, **277**, 48535-49 (2002).

f See, Smart, B.P., et al., *Bioorg. Med. Chem.*, **12**, 1737-1749 (2004).