

Review

# Structure, Function and Regulation of Group IV Phospholipase A<sub>2</sub> Family

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## Abstract

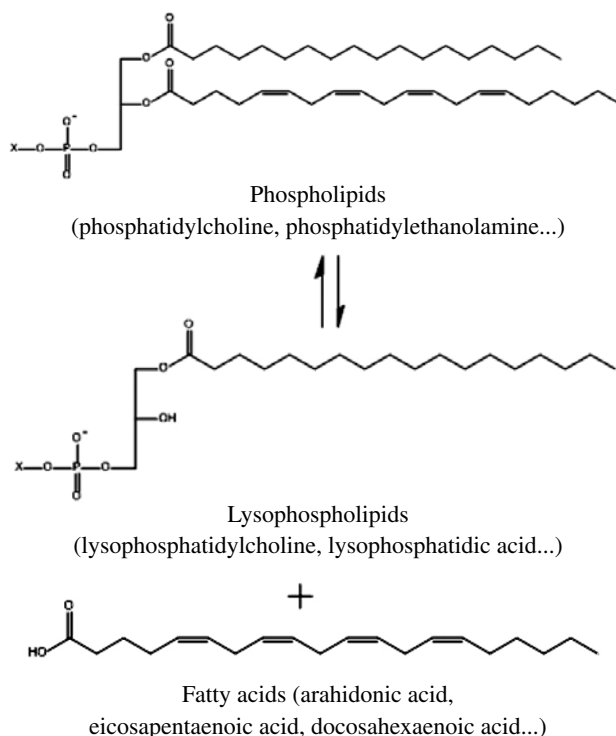
The Group IV phospholipase A<sub>2</sub> family is consisted of six intracellular enzymes. They catalyze hydrolysis of the *sn*-2 ester bond of glycerophospholipids, releasing fatty acid metabolites and lysophospholipids. Agonist-induced release of arachidonic acid for the production of eicosanoids by PLA<sub>2</sub>IValpha enzyme is important in regulating normal and pathological processes in a variety of target tissues. Here, we compare PLA<sub>2</sub>IValpha, and its paralogs β, γ, δ, ε and ζ in term of their structure, function and regulation.

**Keywords:** Group IV phospholipase A<sub>2</sub>; cytosolic phospholipase A<sub>2</sub>; arachidonic acid; C2 domain

## 1. Introduction

PLA<sub>2</sub>s form a superfamily that currently contains 15 separate groups and numerous subgroups of PLA<sub>2</sub> as shown in table 1.<sup>1,2,3</sup> Enzymes are assigned to these groups based on sequence, number of disulfide bonds, molecular weight, calcium requirement, specific substrate and cell localization<sup>3</sup>. The superfamily of PLA<sub>2</sub> comprises a number of vary different proteins that can be divided into five principal kinds of enzymes, the small secreted PLA<sub>2</sub>s (sPLA<sub>2</sub>s), the cytosolic PLA<sub>2</sub>s (cPLA<sub>2</sub>s), the Ca<sup>2+</sup>-independent PLA<sub>2</sub>s (iPLA<sub>2</sub>s), the Platelet-Activating Factor acetylhydrolases (PAF-AHs) and the lysosomal PLA<sub>2</sub>s (LPLA<sub>2</sub>s)<sup>1</sup>. These enzymes are characterized by their ability to specifically hydrolyse the *sn*-2 ester bond of phospholipid substrate, to produce free fatty acids and lysophospholipids, as shown in Fig. 1. Both products represents precursors for signaling molecules that can exert a variety of biological functions.<sup>4</sup>

This review aims to introduce the group IV of PLA<sub>2</sub> enzymes, their structure, biological function, regulation and role in pathophysiological processes, as well as focusing on one well defined mammalian enzyme called group IVA PLA<sub>2</sub> or PLA<sub>2</sub>IValpha or cPLA<sub>2</sub>α. With the completion of the mouse and human genomes it became clear that these mammals also contain other proteins homologous to cPLA<sub>2</sub>α, namely the β, γ, δ, ε and ζ isoforms (groups IVB-F PLA<sub>2</sub>s).<sup>5,6</sup>



**Fig 1:** Chemical reaction catalyzed by the PLA<sub>2</sub> enzymes. Phospholipid is hydrolyzed at the *sn*-2 position to yield free fatty acids and lysophospholipids.

Tabele 1: Phospholipases A<sub>2</sub>

Group	Source	Molecular Mass (kDa)	Type of enzyme
IA	Cobras and kraits	13–15	sPLA <sub>2</sub>
IB	Human/murine	13–15	sPLA <sub>2</sub>
IIA	Rattlesnakes	13–15	sPLA <sub>2</sub>
IIB	Gaboon viper	13–15	sPLA <sub>2</sub>
IIC	Rat/murine	15	sPLA <sub>2</sub>
IID	Human/murine	14–15	sPLA <sub>2</sub>
IIE	Human/murine	14–15	sPLA <sub>2</sub>
IIF	Human/murine	16–17	sPLA <sub>2</sub>
III	Human/murine	55	sPLA <sub>2</sub>
IVA	Human/murine	85	cPLA <sub>2</sub>
IVB	Human	114	cPLA <sub>2</sub>
IVC	Human	61	cPLA <sub>2</sub> *
IVD	Human/murine	92–93	cPLA <sub>2</sub>
IVE	Murine	100	cPLA <sub>2</sub>
IVF	Murine	96	cPLA <sub>2</sub>
V	Human/murine heart/lung/macrophage	14	sPLA <sub>2</sub>
VIA-1	Human/murine	84–85	iPLA <sub>2</sub>
VIA-2	Human/murine	88–90	iPLA <sub>2</sub>
VIB	Human/murine	88–91	iPLA <sub>2</sub>
VIC	Human/murine	146	iPLA <sub>2</sub>
VID	Human	53	iPLA <sub>2</sub>
VIE	Human	57	iPLA <sub>2</sub>
VIF	Human	28	iPLA <sub>2</sub>
VIIA	Human, murine, porcine, bovine	45	PAF-AH
VIIIB	Human, bovine	40	PAF-AH
VIIIA	Human	26	PAF-AH
VIIIB	Human	26	PAF-AH
IX	Snail venom (conodipine-M)	14	sPLA <sub>2</sub>
X	Human spleen/thymus/leukocyte	14	sPLA <sub>2</sub>
XIA	Green rice shoots (PLA <sub>2</sub> -I)	12.4	sPLA <sub>2</sub>
XIB	Green rice shoots (PLA <sub>2</sub> -II)	12.9	sPLA <sub>2</sub>
XII	Human/murine	19	sPLA <sub>2</sub>
XIII	Parvovirus	<10	sPLA <sub>2</sub>
XIV	Symbiotic fungus/ bacteria	13–19	sPLA <sub>2</sub>
XV	Human, murine, bovine	45 (deglycosylated)	LPLA <sub>2</sub>

\* On the basis of sequence similarities, membrane bound group IVC PLA<sub>2</sub> is part of the cytosolic PLA<sub>2</sub>s enzymes

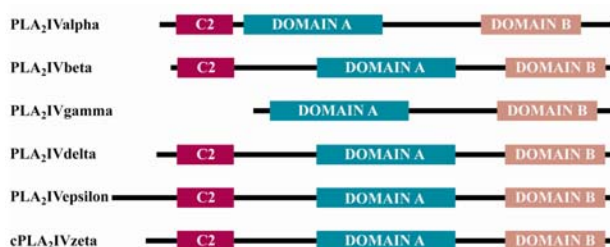
## 2. Properties of Group IVA PLA<sub>2</sub> (PLA<sub>2</sub>IValpha)

PLA<sub>2</sub>IValpha plays a very important role in the release of arachidonic acid, a regulator of diverse cellular functions and a precursor for biosynthesis of potent inflammatory lipids such as eicosanoids, including prostaglandins, thromboxanes, leukotrienes and lipoxins<sup>7</sup>. PLA<sub>2</sub>IValpha shows almost no homology with other PLA<sub>2</sub>s. Among all PLA<sub>2</sub>s, receptor-mediated arachidonic release is primarily attributed to PLA<sub>2</sub>IValpha. It preferentially hydrolyses arachidonic acid from the *sn*-2 position of membrane phospholipids in enzyme reaction that occurs in many cell types in response to varieties of extracellular stimuli<sup>8,9,10</sup>. PLA<sub>2</sub>IValpha is on human chromosome 1 (mouse chromosome 1)<sup>11</sup>. It is highly conserved through

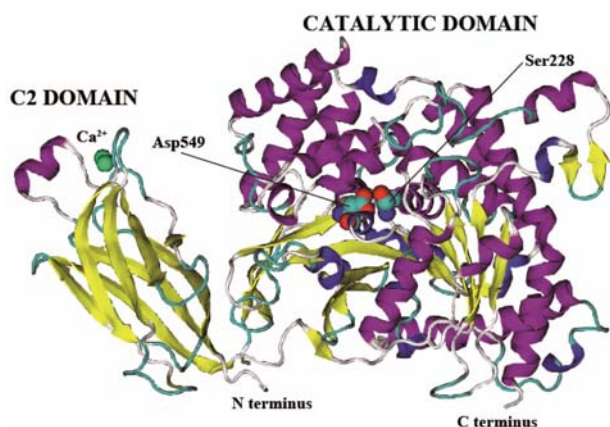
evolution with human and mouse homologues sharing over 95% amino acid identity<sup>10</sup>. The homologues found in chickens, zebra fish and xenopus have over 80% amino acid identity with human PLA<sub>2</sub>IValpha, consistent with an important conserved functional role. On the contrary, the mammalian Group IV paralogs PLA<sub>2</sub>s β, γ, δ, ε and ζ genes are less conserved and share approximately 30–37% amino acid identity with PLA<sub>2</sub>IValpha, suggesting different role in the cell.<sup>5</sup>

The PLA<sub>2</sub>IValpha cDNA encodes a protein with a molecular weight of 85 kDa.<sup>10</sup> Primary structure of this enzyme starts with N-terminal calcium-dependent lipid binding domain (C2 or CaLB) followed by a catalytic domain (Fig. 2, Fig. 3).<sup>10,12</sup> The group IV PLA<sub>2</sub> family does not contain a classical lipase catalytic domain composed of a serine/acid/histidine triad. Instead, catalytic center is utilizing a conserved serine/aspartic acid dyad.<sup>12</sup> Recent

kinetic study of PLA<sub>2</sub>IValpha revealed that enzyme displays a PLA<sub>1</sub>/PLA<sub>2</sub> specific activity ratio of 0.1 showing that it is PLA<sub>2</sub> enzyme. PLA<sub>2</sub>IValpha displays relatively high lysophospholipase activity as well.<sup>13</sup> With the exception of PLA<sub>2</sub>IVgamma all members of the group IV contain a C2 domain (Fig. 2). This hydrophobic C2 domain function to promote interaction of proteins with membranes.<sup>14</sup> It is composed approximately 120 amino acids that fold in an eight-stranded anti-parallel  $\beta$ -sheet. C2 domain binds 2-3 ions of calcium through aspartatic acid residues. Calcium binding neutralizes the anionic residues and favours PLA<sub>2</sub>IValpha interaction with the membranes.<sup>15</sup>



**Fig 2:** Schematic representation of primary structures of murine group IV of PLA<sub>2</sub> enzymes. Length of lines represents the polypeptide lengths, and boxes represent conserved domains with similarity within group IV of PLA<sub>2</sub>s. C2 domain: calcium binding domain; catalytic domain A: the lipase consensus sequence, GXSGS, is located in its N-terminal; catalytic domain B: the lipase consensus sequence, DxG (except DTA in PLA<sub>2</sub>Ivepsilon).



**Fig 3:** Structural features of human PLA<sub>2</sub>IValpha. The cartoon diagram of human PLA<sub>2</sub>IValpha shows  $\alpha$ -helices (purple) and  $\beta$ -sheets (yellow). C2 domain binds two calcium ions (green) and promotes an enzyme to translocate from the cytosol to the membranes. In the catalytic domain, residues essential for catalytic activity including the Ser228/Asp549 dyad are shown in active site.

PLA<sub>2</sub>IValpha is a widely distributed enzyme which is constitutively expressed in most cells and tissues.<sup>8,16</sup> Expression of PLA<sub>2</sub>IValpha is increased by certain cytokines or inhibited by glucocorticoids.<sup>8</sup> Regulation of PLA<sub>2</sub>IValpha by activators or signal transducers can in-

volve effects on mRNA stability and transcription.<sup>17,18</sup> PLA<sub>2</sub>IValpha is regulated by physiological levels of intracellular calcium concentration and phosphorylation by mitogen-activated protein kinase (MAPK)<sup>10,19,20</sup>. PLA<sub>2</sub>IValpha is activated at submicromolar rather than millimolar calcium concentration<sup>21,22</sup>. Calcium binds to C2 domain and induces translocation of PLA<sub>2</sub>IValpha from cytosol to the membranes of Golgi, endoplasmic reticulum, nuclear envelope<sup>23,24,25,26</sup> and less frequently to the plasma membranes<sup>27</sup> or inside of the nucleus<sup>28</sup>. This is an important step in regulation of enzyme to access its substrate. However, translocation of PLA<sub>2</sub>IValpha can occur without increase in calcium, indicating alternative regulatory mechanisms<sup>23</sup>. The MAPK family includes several subgroups including extracellular signal-regulated kinases, ERK1 (p44) and ERK2 (p42); c-Jun N-terminal kinases (JNKs) and p38. Agonist-induced phosphorylation of PLA<sub>2</sub>IValpha at serine 505 by ERKs increases its catalytic activity and results in characteristic gel shift<sup>19,26,29</sup>. Although the PLA<sub>2</sub>IValpha phosphorylation by ERKs<sup>19,26,29</sup> and p38<sup>30,31,32</sup> is well documented in the literature, only few reports indicate possible involvement of JNKs in PLA<sub>2</sub>IValpha phosphorylation<sup>32,33</sup>. Beside PLA<sub>2</sub>IValpha regulation by protein kinases and calcium, there is also evidence for direct association C2 domain with other binding proteins such as vimetin, which augments arachidonic acid release<sup>34</sup>. Activated receptors mediate signal via heterotrimeric GTP-binding (G) proteins to their effector enzymes, which include several phospholipases<sup>19,35</sup>. In particular, the G<sub>o</sub>/G<sub>i</sub> and G<sub>q</sub> protein families have been shown to couple signaling to PLA<sub>2</sub>IValpha<sup>36,37</sup>. Studies on dominant negative G<sub>12</sub> mutant demonstrated that inhibition of thrombin and ATP stimulated PLA<sub>2</sub>IValpha mediated arachidonic release is MAPK and calcium independent, indicating possible direct coupling G-proteins with PLA<sub>2</sub>IValpha<sup>36,38</sup>. We demonstrated that in CHO cells, the G<sub>12</sub>/G<sub>13</sub> family is also able to activate PLA<sub>2</sub>IValpha, through the activation of RhoA and, subsequently, ERK1/2<sup>39</sup>.

### 3. Properties of Group IVB PLA<sub>2</sub> (PLA<sub>2</sub>IVbeta)

Human PLA<sub>2</sub>IVbeta was cloned several years ago<sup>40,41</sup>, but little is known about its structure, function and regulation. PLA<sub>2</sub>IVbeta gene is located on chromosome 15. PLA<sub>2</sub>IVbeta mRNA is expressed ubiquitously in human but more highly in cerebellum, heart, and pancreas<sup>41</sup>. More specifically, in rat cerebellum PLA<sub>2</sub>IVbeta is localized in granule cells<sup>42</sup>. The form of PLA<sub>2</sub>IVbeta cDNA encodes a protein with a predicted molecular weight of 114 kDa<sup>40</sup>. Catalytic and C2 domains of PLA<sub>2</sub>IValpha and PLA<sub>2</sub>IVbeta share about 30% amino acid identity. Human PLA<sub>2</sub>IVbeta contains a unique N-terminal 242 amino acid extension upstream of the C2 domain that con-

tains a partial JmjC domain, but mouse PLA<sub>2</sub>IVbeta does not. JmjC domains are predicted to be metalloenzymes that are often found in nuclear proteins and contain DNA and/or chromatin binding motifs, and regulate chromatin stability<sup>43</sup>. The truncated JmjC in PLA<sub>2</sub>IVbeta would not be predicted to have a similar function since its structure is not complete. However, the truncated JmjC domain of PLA<sub>2</sub>IVbeta may affect its membrane binding and/or catalytic properties although this remains to be determined. It is obvious that PLA<sub>2</sub>IVbeta mRNA undergoes complex transcriptional and splicing regulation resulting in the production of functionally diverse protein products. PLA<sub>2</sub>IVbeta was found to be expressed as a 100 kDa protein in human tissues and in a human lung epithelial cell line (BEAS-2B), not the 114 kDa protein originally predicted<sup>44</sup>. BEAS-2B cells contain three PLA<sub>2</sub>IVbeta splice variants (PLA<sub>2</sub>IVβ1, β2 and β3). All three transcripts contain the truncated JmjC domain and the C2 domain, but have differences in the catalytic domain. PLA<sub>2</sub>IVβ1 is identical to the originally cloned form, whereas PLA<sub>2</sub>IVβ2 and PLA<sub>2</sub>IVβ3 contain internal deletions in the catalytic domain resulting in smaller proteins of 100 kDa. However, only PLA<sub>2</sub>IVβ3 is translated into protein in BEAS-2B cells<sup>44</sup>. Although PLA<sub>2</sub>IVβ3 exhibits calcium-dependent PLA<sub>2</sub> activity, it was found to be constitutively associated with membrane in BEAS-2B cells, and localizes to mitochondria and early endosomes<sup>44</sup>. PLA<sub>2</sub>IVβ3 is widely expressed in tissues suggesting that it plays a generalized role at these organelles. A recent kinetic study of PLA<sub>2</sub>IVbeta revealed that enzyme displays a PLA<sub>1</sub>/PLA<sub>2</sub> specific activity ratio of 1.3 showing that it is a dual PLA<sub>1</sub>/PLA<sub>2</sub> enzyme. PLA<sub>2</sub>IVbeta displays relatively high lysophospholipase activity as well<sup>13</sup>. PLA<sub>2</sub>IVbeta is an important metabolic enzyme not only in mammals but also in plants, where regulates light-induced stomatal opening in Arabidopsis<sup>45</sup>.

#### 4. Properties of Group IVC PLA<sub>2</sub> (PLA<sub>2</sub>IVgamma)

Like PLA<sub>2</sub>IVbeta, human PLA<sub>2</sub>IVgamma was cloned several years ago<sup>40,46</sup> but little is known about its structure and function. PLA<sub>2</sub>IVgamma gene is located on chromosome 19. Unlike PLA<sub>2</sub>IValpha, human PLA<sub>2</sub>IVgamma mRNA is not ubiquitous. PLA<sub>2</sub>IVgamma mRNA is expressed most strongly in skeletal muscle and heart. The form of PLA<sub>2</sub>IVgamma cDNA encodes a protein with a molecular weight of 61 kDa<sup>40</sup>. Human PLA<sub>2</sub>IVgamma is 30% homologous to PLA<sub>2</sub>IValpha and the residues necessary for PLA<sub>2</sub>IValpha catalytic activity are conserved in PLA<sub>2</sub>IVgamma<sup>40</sup>. PLA<sub>2</sub>IVgamma lacks both the regulatory phosphorylation sites present in PLA<sub>2</sub>IValpha and a C2 domain, but contains a prenyl group-binding site motif<sup>46</sup>. Farnesylation of C-terminal of the PLA<sub>2</sub>IVgamma, together with palmitoylation<sup>47</sup> en-

hance protein hydrophobicity and most likely facilitate its localization to the endoplasmic reticulum, Golgi apparatus<sup>48</sup> and mitochondria<sup>47</sup>. This calcium independent enzyme displays a PLA<sub>1</sub>/PLA<sub>2</sub> specific activity ratio of 0.2 showing that it is mainly PLA<sub>2</sub> enzyme. PLA<sub>2</sub>γ also displays lysophospholipase activity on <sup>14</sup>C-P-LPC comparable to that of PLA<sub>2</sub>IVbeta and PLA<sub>2</sub>IValpha<sup>13</sup>. In some cases, PLA<sub>2</sub>IVgamma may also function as a transacylase and consequently may play a role in phospholipid remodeling<sup>49</sup>. Furthermore, H<sub>2</sub>O<sub>2</sub> and other hydroperoxides induce arachidonic acid release in PLA<sub>2</sub>IVgamma, suggesting that it may be involved in metabolism of oxidative stress to repair oxidized phospholipids<sup>50</sup>. The arachidonic acid released by PLA<sub>2</sub>IVgamma upon agonist stimulation is metabolized further to prostaglandin E<sub>2</sub> via cyclo-oxygenase-1 (COX-1) in the immediate response, and via COX-2 in the delayed response<sup>48</sup>. In bovine endometrial epithelial cells PLA<sub>2</sub>IVgamma regulates prostaglandin E<sub>2</sub> and F<sub>2</sub>α production upon oxytocin stimulation<sup>51</sup>. PLA<sub>2</sub>IVgamma is also present in human retina, but its function remains unknown<sup>52</sup>. Epithelial PLA<sub>2</sub>IVgamma accounts for the increased lysophospholipase activity observed during intestinal nematodiasis and it plays a major role in the inflammatory response to nematodes<sup>53</sup>. Interestingly, expression of mouse PLA<sub>2</sub>IVgamma is restricted to the oocyte and early embryo, suggesting a unique role for mouse PLA<sub>2</sub>IVgamma in early embryonic development<sup>54</sup>.

#### 5. Properties of Group IVD (PLA<sub>2</sub>IVdelta)

Like PLA<sub>2</sub>IVgamma, human PLA<sub>2</sub>IVdelta was cloned several years ago<sup>55</sup>. PLA<sub>2</sub>IVdelta gene is located on chromosome 15, near PLA<sub>2</sub>IVbeta gene. PLA<sub>2</sub>IVdelta mRNA is uniquely expressed in stratified squamous epithelium of cervix, fetal skin and prostate. cDNA of PLA<sub>2</sub>IVdelta encodes a protein of approximately 90 kD and has greatest homology with PLA<sub>2</sub>IVbeta, PLA<sub>2</sub>IValpha and PLA<sub>2</sub>IVgamma in the C2 and catalytic domain. Human PLA<sub>2</sub>IVdelta has calcium-dependent release of arachidonic acid from 1-palmitoyl-2-[<sup>14</sup>C]arachidonoyl-phosphatidylcholine<sup>55</sup>. It displays a PLA<sub>1</sub>/PLA<sub>2</sub> specific activity ratio of 5.3 showing that it is predominantly PLA<sub>1</sub> enzyme. PLA<sub>2</sub>IVdelta also displays lysophospholipase activity on <sup>14</sup>C-P-LPC comparable to that of PLA<sub>2</sub>IValpha, PLA<sub>2</sub>IVbeta and PLA<sub>2</sub>IVgamma<sup>13</sup>. In humans, PLA<sub>2</sub>IVdelta may play a critical role in inflammation in psoriatic lesions<sup>55</sup>. Murine PLA<sub>2</sub>IVdelta gene is located on chromosome 2, forms gene cluster with PLA<sub>2</sub>IVbeta, PLA<sub>2</sub>IVepsilon and PLA<sub>2</sub>IVzeta gene. PLA<sub>2</sub>IVdelta mRNA is in majority expressed in placenta, unlike the human homologue. The deduced amino acid sequence of PLA<sub>2</sub>IVdelta revealed a C2 domain and catalytic domain. Murine PLA<sub>2</sub>IVdelta is more homologous to PLA<sub>2</sub>IVbeta

(41–50% amino acid identity in the catalytic domain and 33–43% identity in the C2 domain) than to PLA<sub>2</sub>IValpha or PLA<sub>2</sub>IVgamma (30–37% identity in the catalytic domain and 25–28% identity in the C2 domain). Recombinant protein demonstrated molecular weight of about 100 kDa and exhibited calcium dependent PLA<sub>2</sub> activity. Protein is enzymatically active but do not exhibit specificity for *sn*-2 arachidonic acid. PLA<sub>2</sub>IVdelta translocates from cytosol to perinuclear sites in response to calcium ionophore<sup>5</sup>.

## 6. Properties of Group IVE (PLA<sub>2</sub>IVepsilon)

Human PLA<sub>2</sub>IVepsilon has not been cloned yet. But like murine PLA<sub>2</sub>IVdelta, murine PLA<sub>2</sub>IVepsilon was cloned several years ago<sup>5</sup>. Murine PLA<sub>2</sub>IVepsilon gene is located on chromosome 2, forms gene cluster with PLA<sub>2</sub>IVbeta, PLA<sub>2</sub>IVdelta and PLA<sub>2</sub>IVzeta gene. PLA<sub>2</sub>IVepsilon mRNA is predominantly expressed in thyroid, heart, testis and skeletal muscle. From the deduced amino acid sequence of PLA<sub>2</sub>IVepsilon, C2 domain and catalytic domain has been determined. Both domains are more homologous to PLA<sub>2</sub>IVbeta than to PLA<sub>2</sub>IValpha or PLA<sub>2</sub>IVgamma, similarly as seen in murine PLA<sub>2</sub>IVdelta. Recombinant protein demonstrated molecular weight of about 100 kDa. It requires calcium for its activity. Protein is enzymatically active but do not exhibit specificity for *sn*-2 arachidonic acid. PLA<sub>2</sub>IVepsilon appears to be partly associated with lysosomes, but not with ER/Golgi or mitochondria. Stimulation with ionomycin did not cause redistribution of PLA<sub>2</sub>IVepsilon in cytosol<sup>5</sup>. Recently same synthetic genes coding for human PLA<sub>2</sub>IVepsilon were prepared and kinetic studies on recombinant protein were determined. Enzyme displays only a PLA<sub>1</sub> specific activity, which is relatively low compared to other cPLA<sub>2</sub>s. This human PLA<sub>2</sub>IVepsilon has extremely low specific activity as a lysophospholipase on <sup>14</sup>C-P-LPC and as a PLA<sub>2</sub> on <sup>14</sup>C-PAPC vesicles<sup>13</sup>.

## 7. Properties of Group IVF (PLA<sub>2</sub>IVzeta)

Human PLA<sub>2</sub>IVzeta has not been cloned yet. Like murine PLA<sub>2</sub>IVdelta and PLA<sub>2</sub>IVepsilon, murine PLA<sub>2</sub>IVzeta was cloned several years ago<sup>5</sup>. Murine PLA<sub>2</sub>IVzeta gene is located on chromosome 2 and it is part of a gene cluster containing PLA<sub>2</sub>IVbeta, PLA<sub>2</sub>IVdelta and PLA<sub>2</sub>IVepsilon. PLA<sub>2</sub>IVzeta mRNA is expressed in thyroid and stomach. Both, C2 domain and catalytic domain are more homologous to PLA<sub>2</sub>IVbeta than to PLA<sub>2</sub>IValpha or PLA<sub>2</sub>IVgamma, similarly as seen in murine PLA<sub>2</sub>IVdelta and PLA<sub>2</sub>IVepsilon. Recombinant protein with a molecular weight of about 100 kDa requires

calcium for its activity. Enzyme does not exhibit specificity for *sn*-2 arachidonic acid. It displays a PLA<sub>1</sub>/PLA<sub>2</sub> specific activity ratio of 0.6 showing that it is predominantly PLA<sub>2</sub> enzyme. PLA<sub>2</sub>IVzeta displays very high lysophospholipase activity on <sup>14</sup>C-P-LPC comparable to that of PLA<sub>2</sub>IValpha, and PLA<sub>2</sub>IVbeta<sup>13</sup>. PLA<sub>2</sub>IVzeta is cytosolic in resting cells and does not translocate to membranes after addition of ionomycin in CHO-K1 cells<sup>5</sup>. PLA<sub>2</sub>IVzeta exhibits specific activity, inhibitor sensitivity, and low micromolar calcium dependence similar to PLA<sub>2</sub>IValpha but different sublocalization in mouse lung fibroblasts. In response to ionomycin, EGFP-PLA<sub>2</sub>IVzeta translocated to ruffles and dynamic vesicular structures<sup>56</sup>.

## 8. Physiological and Pathological Roles of cPLA<sub>2</sub>

The PLA<sub>2</sub>IValpha knockout mouse model has provided important information about its role in physiological processes and disease.<sup>80</sup> The normal phenotype of PLA<sub>2</sub>IValpha-null mice suggested that this enzyme is not crucial for development and normal physiology<sup>59</sup>. However, when the mice were tested for various diseases, especially those involving inflammation, the symptoms were much milder than wild type mice. PLA<sub>2</sub>IValpha-null mice showed a great reduction in lipid mediator production which led to resistance to ischemia–reperfusion injury<sup>60</sup>, anaphylactic responses<sup>61</sup> acute respiratory distress syndrome caused by acid or endotoxin<sup>62,63</sup>, bleomycin-induced pulmonary fibrosis<sup>64</sup>, collagen-induced autoimmune arthritis<sup>65</sup>, experimental allergic encephalomyelitis<sup>66</sup>. In tumorigenesis of small intestine, PLA<sub>2</sub>IValpha may have a role in the expansion of polyps rather than the initiation process<sup>67</sup>. Concerning normal physiology, the loss of PLA<sub>2</sub>IValpha causes some defects on the renal concentrating function, have ulcerative lesions in the small intestine, enlarged hearts and defects in female reproduction implicating PLA<sub>2</sub>IValpha and its metabolites in regulating normal physiological processes<sup>68,69,70,71,72,73</sup>. Activation of PLA<sub>2</sub>IValpha is essential for thrombin induced smooth muscle cell proliferation, which might lead to pathological thickening of vascular walls in atherosclerosis<sup>74</sup>. There is very little published evidence on the role of PLA<sub>2</sub>IValpha in animal models of atherosclerosis. But Wyeth suggests that apoE–/–, PLA<sub>2</sub>IValpha ko mice showed a reduced atherosclerotic plaque burden<sup>75</sup>. There are also some reports on PLA<sub>2</sub>IValpha which might play a role in severe asthma pathogenesis<sup>76</sup>. Recognition of the importance of the PLA<sub>2</sub>IValpha in inflammatory diseases has made it a very attractive drug target. Two of the most promising drug candidates include the indole derivative inhibitors developed by Wyeth<sup>77,78</sup>, which display anti-arthritis and anti-bone destructive action<sup>79</sup>, and prevent experimental

autoimmune encephalomyelitis<sup>80</sup>, and the 2-oxoamide inhibitors developed by Six<sup>81</sup> and McKew<sup>82</sup>, which show potency in reducing inflammatory effects<sup>82</sup>. There is also a report on a series of ketone-containing compounds that are also potent inhibitors of PLA<sub>2</sub>IValpha<sup>83,84</sup>. The Wyeth are developing a new PLA<sub>2</sub>IValpha inhibitor, girapladib, for osteoarthritis but data are limited<sup>75</sup>.

Among many PLA<sub>2</sub>s, PLA<sub>2</sub>IValpha plays a central role in the lipid mediator production in pathological conditions, and therefore, we propose that PLA<sub>2</sub>IValpha can be a potential target for the development of a novel class of non-steroidal anti-inflammatory drugs. Different biochemical approaches in vivo<sup>59</sup> and in vitro<sup>85,86,87</sup> must be carefully used to elucidate the role of the group IV PLA<sub>2</sub> in normal and pathological physiology.

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## Povzetek

Skupina IV fosfolipaz  $A_2$  trenutno obsega šest encimov. Encimi katalizirajo hidrolizo *sn*-2 estrske vezi glicerofosfolpidov, pri čemer nastane maščobna kislina in lizofosfolipid. Največkrat je produkt hidrolize arahidonska kislina, ki se pretvarja v aktivne eikozanoide, ki imajo pomembno vlogo pri regulaciji celičnih procesov v različnih tarčnih tkivih. V tem prispevku si bomo pogledali strukturo, funkcijo in regulacijo encima  $PLA_2IV\alpha$  in jo primerjali z ostalimi predstavniki skupine IV fosfolipaz  $A_2$ , in sicer z  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$  and  $\zeta$ .