Biochemical and Biophysical Characterization of Human Secretory Phospholipases

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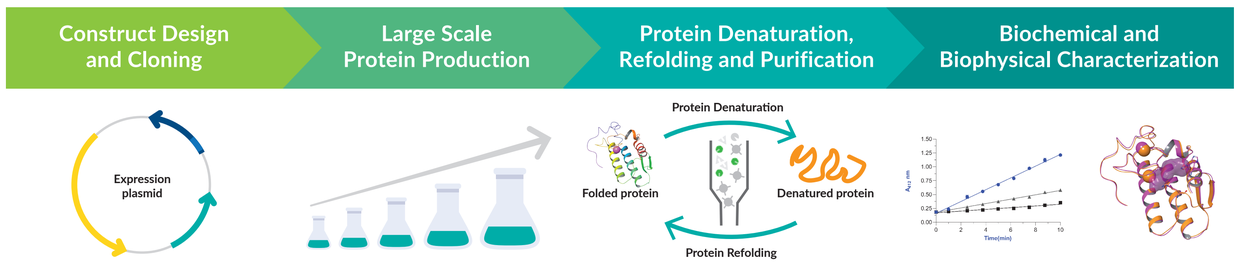
KEY FINDING

sPLA$_2$ enzyme activity was compared in the presence and absence of inhibitors. sPLA$_2$- IIA apo and inhibitor-bound structures were solved with 1.72 Å and 1.68 Å resolutions, respectively.

INTRODUCTION

Secretory phospholipase A$_2$ (sPLA$_2$) catalyzes the hydrolysis of *sn-2* acyl bond of membrane-bound phospholipids yielding a free fatty acid and a lysophospholipid. The sPLA$_2$ family contains 10 catalytically active (IB, IIA, IID, IIE, IIF, III, V, X, and XIIA) and one inactive isoform (XIIB) in mammals.[1,2,3] This family of enzymes are disulfide-rich, low molecular weight, Ca^{2+}-requiring lipolytic enzymes with a His-Asp catalytic dyad. Individual mammalian sPLA$_2$s have distinct enzymatic properties and display distinct tissue expression patterns, suggesting that each enzyme acts on a distinct phospholipid membrane.[3] Here we describe the comparative activity of sPLA$_2$-IIA, sPLA$_2$-IID, sPLA$_2$-IIE, sPLA$_2$-V, and sPLA$_2$-X in the presence and absence of inhibitors. The closely related isoforms (sPLA$_2$-IIA, sPLA$_2$-IID, and sPLA$_2$-IIE) showed preference for dihepatanoyl thio-PE (Thio-PE), whereas the other isoforms (sPLA$_2$-V and sPLA$_2$-X) preferred dihepatanoyl thio-PC (Thio-PC) as a substrate. Inhibition assays showed that varespladib and AZD2716 inhibited enzyme activity. In the presence of 100 nM varespladib, Thio-PC conversion was abolished for all the sPLA$_2$s except for sPLA$_2$-V. However, 100 nM AZD2716 completely inhibited Thio-PC activity for sPLA$_2$-V, sPLA$_2$-IID and sPLA$_2$-IIE followed by residual (~<20%) activity for sPLA$_2$-IIA and sPLA$_2$-X. In contrast, 100 nM varespladib showed 100% inhibition for sPLA$_2$-X and sPLA$_2$-IIA in the presence of Thio-PE. In addition, we have solved the crystal structures of sPLA$_2$-IIA, apo and inhibitors bound with 1.72 Å and 1.68 Å resolutions, respectively.

EXPERIMENTAL WORKFLOW



RESULTS

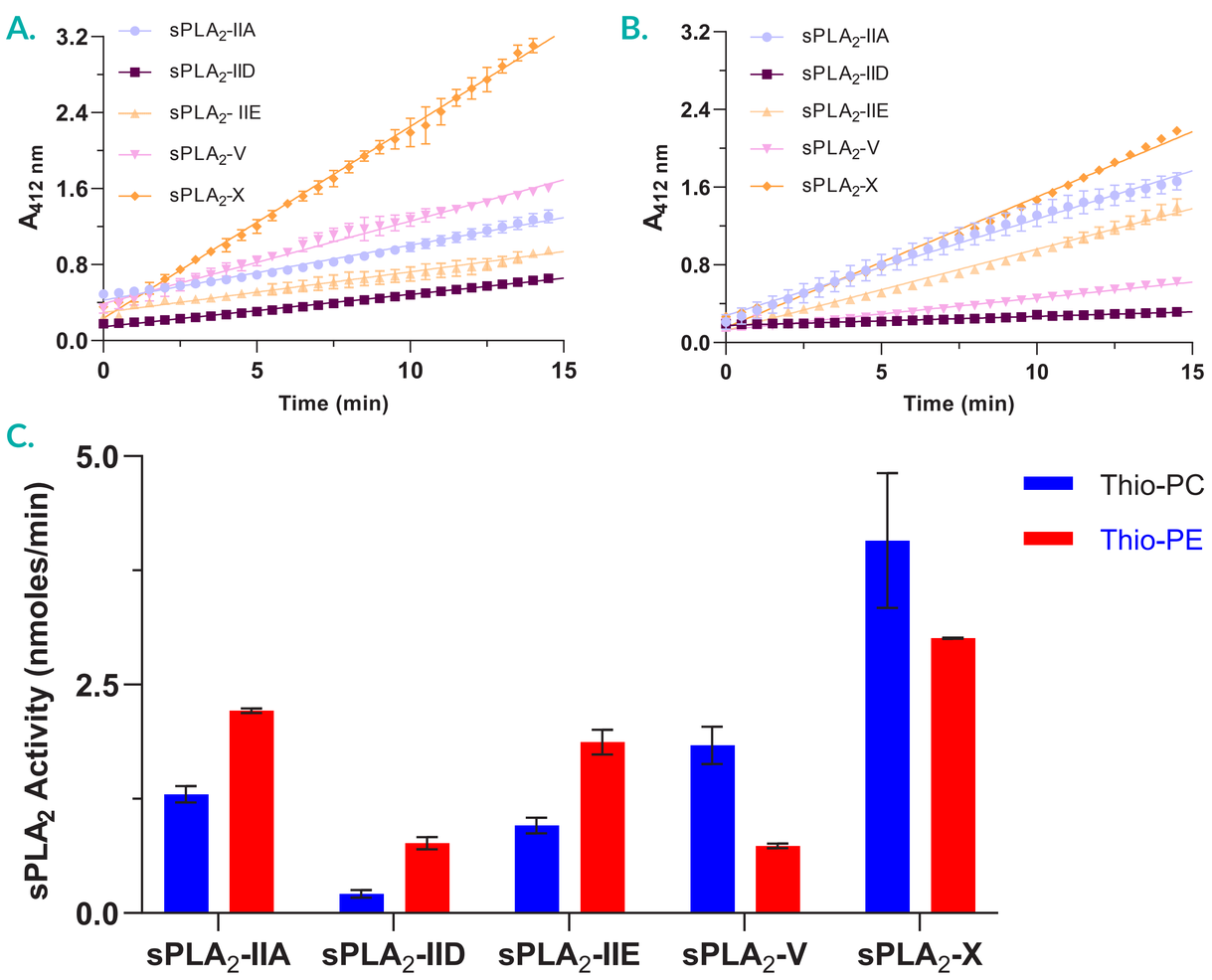


FIGURE 1 – sPLA$_2$ enzyme substrate specificity.
sPLA$_2$ enzyme activity was determined using Cayman's sPLA$_2$ Assay Kit (Item No. 765001) with **A)** 1.48 mM Thio-PC (Cayman Item No. 62235) and **B)** 1.48 mM Thio-PE (Cayman Item No. 38245) as substrate. 200 ng of sPLA$_2$ enzyme was used in all reactions and assay was conducted for 15 minutes. **C)** Substrate preference for the sPLA$_2$ enzyme activity. sPLA$_2$-V and sPLA$_2$-X prefer Thio-PC as substrate, and sPLA$_2$-IIA, sPLA$_2$-IID, and SPLA$_2$-IIE prefer Thio-PE as substrate.

References

1. Dennis, E.A. Diversity of group types, regulation and function of phospholipase A2. *J. Biol. Chem.* **269**, 13057-13060 (1994).
2. Murakami *et al.* Secreted phospholipase A2 revisited. *J. Biol. Chem.* **150**, 233-255 (2011).
3. Murakami *et al.* A new era of secreted phospholipase A2. **56**, 1248-1261 (2015).

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RESULTS CONT.

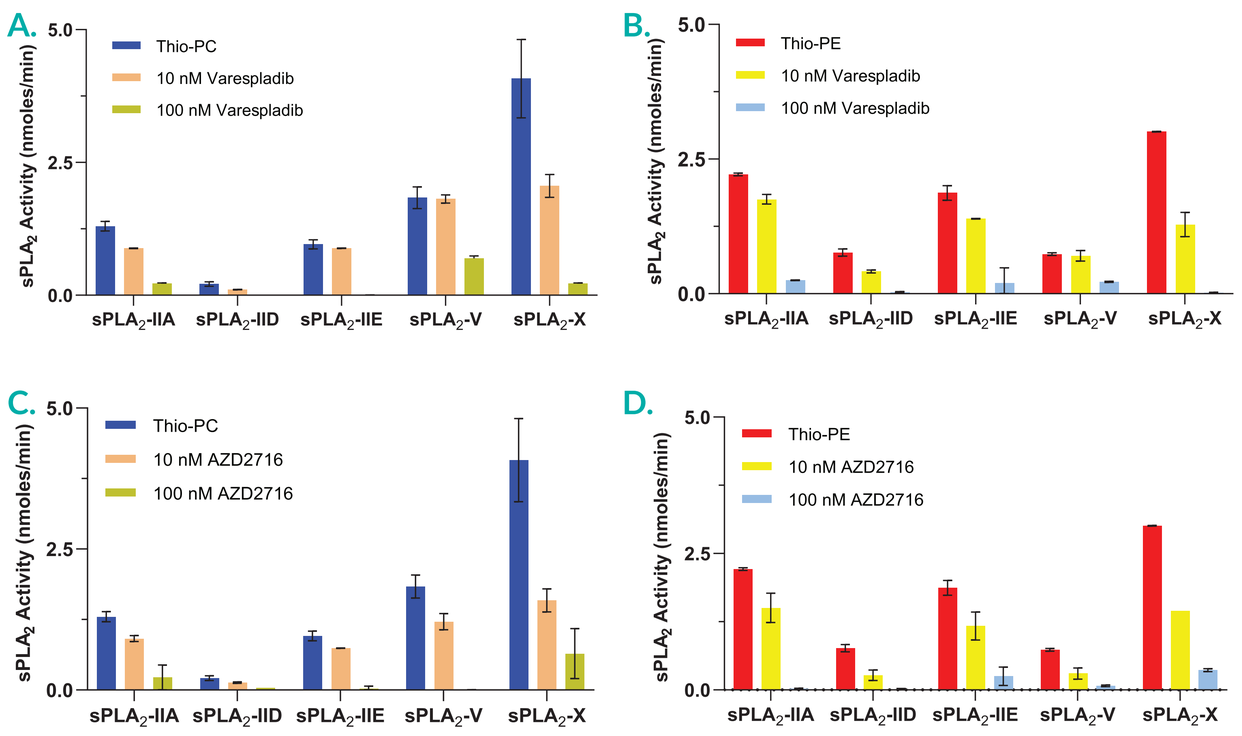

FIGURE 2 – sPLA$_2$ enzyme activity in the presence of the inhibitor.
200 ng of sPLA$_2$ was used in the assay with 10 nM and 100 nM of varespladib (Cayman Item No. 18267) and AZD2716 sPLA$_2$ inhibitors (Cayman Item No. 35067). **A)** and **B)** sPLA$_2$ enzyme activity inhibited by varespladib. sPLA$_2$ activity was measured in the presence of 10 nM and 100 nM varespladib in the presence of Thio-PC and Thio-PE, respectively. **C)** and **D)** sPLA$_2$ enzyme activity inhibited by AZD2716. sPLA$_2$ activity was measured in the presence of 10 nM and 100 nM AZD2716 in the presence of Thio-PC and Thio-PE, respectively.

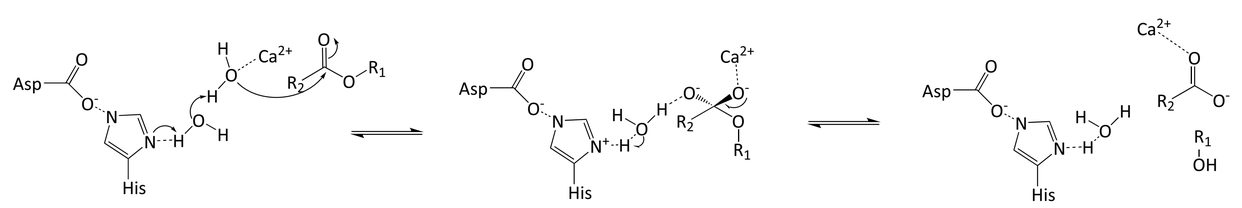

FIGURE 3 – Ca^{2+}-dependent sPLA$_2$ enzyme reaction mechanism.
The water molecule is activated by the presence of a histidine/aspartic acid dyad in a Ca^{2+}-dependent manner.

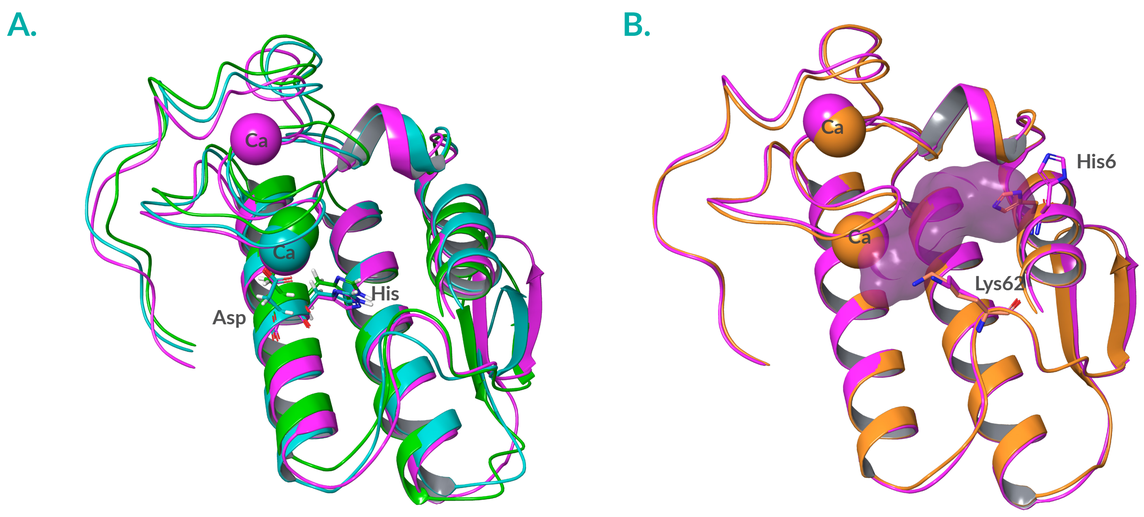

FIGURE 4 – Structure comparison of sPLA$_2$ enzymes.
A) Overlay with ribbon model of sPLA$_2$-IIA (1.68 Å crystal structure solved by Cayman SBBC (unpublished) (magenta), sPLA$_2$-IIE (5WZW.pdb)(cyan), and sPLA$_2$X (6G5J.pdb)(green). Calcium is shown as sphere in corresponding color. Catalytic His and Asp residues in active site shown in the corresponding colors as stick models. **B)** Overlay with ribbon models of sPLA$_2$-IIA complexed with inhibitor shown as space filling model (1.68 Å crystal structure solved by Cayman SBBC (unpublished) (magenta).

TABLE 1 – Variables obtained from SPR analysis using Biacore™ Insight software using varespladib inhibitor.

	sPLA$_2$-IIA	sPLA$_2$-X
1:1 binding k_a (M^{-1} s^{-1})	2.05e+06	8.21e+05
k_d (s^{-1})	2.01e-02	1.65e-03
K_D (M)	9.77e-09	2.02e-09

CONCLUSIONS

- sPLA$_2$-IIA, sPLA$_2$-IID, sPLA$_2$-IIE, sPLA$_2$-V, and sPLA$_2$-X enzymes were purified from inclusion bodies and refolded.
- sPLA$_2$-IIA, sPLA$_2$-IID, sPLA$_2$-IIE preferred Thio-PE, and sPLA$_2$-V, and sPLA$_2$-X preferred Thio-PC as substrate.
- Varespladib and AZD2716 inhibited sPLA$_2$ enzyme activity.
- Crystal structures of sPLA$_2$-IIA, apo and inhibitor bound were solved with 1.72 Å and 1.68 Å resolution, respectively.