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Review

Distinguishing phospholipase A_2 types in biological samples by employing group-specific assays in the presence of inhibitors

Karin Killermann Lucas, Edward A. Dennis *

University of California, San Diego School of Medicine, Department of Chemistry, Biochemistry and Pharmacology, 9500 Gilman Drive, La Jolla, San Diego, CA 92093 0601, USA

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Abstract

This manuscript reviews and updates radiolabel-based enzyme assays designed to distinguish the activity of phospholipase A_2 (PLA_2) types in biological samples. This approach should be useful in lipidomics studies. The assays were originally designed to differentiate between Group IVA cytosolic PLA_2 (GIVA $cPLA_2$), Group VIA calcium-independent PLA_2 (GVIA $iPLA_2$), Group IIA secreted PLA_2 (GIIA $sPLA_2$) and Group V secreted PLA_2 (GV $sPLA_2$). The specificity of these assays has now been confirmed using purified, recombinant human PLA_2s and the utility of these assays is demonstrated with rat spinal cord homogenate as an example of a biological tissue sample of interest to the neuroscience community. Modifications to the original assays by the addition of group-specific inhibitors are presented to ensure the specificity of the assays and to further differentiate between recently identified PLA_2s. Specific tests are suggested to confirm the specificity of each assay. Additionally, it was discovered that one commonly used GIVA $cPLA_2$/GVIA $iPLA_2$ inhibitor, methyl arachidonyl fluorophosphonate (MAFP) from one commercial source, was found to inhibit GIIA $sPLA_2$ and GV $sPLA_2$, but not GIVA $cPLA_2$, presumably due to oxidation of the compound during shipment, resulting in a different molecule with altered specificity.
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Keywords: Lipidomics; Phospholipase A_2; MAFP; BEL; Indoxam; Dole assay; PLA_2 activity assay; PIP_2

* Corresponding author. Tel.: +1 858 534 3055; fax: +1 858 534 7390.
E-mail address: edennis@ucsd.edu (E.A. Dennis).

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Contents

1. Introduction

Phospholipase A_2 (PLA_2) catalyzes the hydrolysis of fatty acids from the *sn*-2 position of phospholipids. The release of fatty acids including arachidonic acid (AA) and lysophospholipids from the cell membrane by PLA_2 activity leads to a cascade of lipid second messengers that regulates a wide variety of physiological responses. Interest in the PLA_2 field has increased in the past decade with the discovery of the important role of lipid messengers in diseases such as cancer [1–3] and atherosclerosis [4,5]. While lipidomics approaches should help elucidate the intricate relationships of the numerous lipid messengers, the ability to also block and measure the activity of the specific PLA_2 enzymes that initiate lipid second messenger release should aid in lipidomics studies.

In 1999, our laboratory published a manuscript on group-specific assays describing four specific PLA_2 assays [6] that were designed to distinguish between the four major mammalian PLA_2 enzymes that had been identified at that time. The assays took advantage of differences in preferred lipid substrate and activators, calcium dependence and susceptibility to disulfide bond reduction to distinguish between the Group IVA cytosolic PLA_2 (GIVA $cPLA_2$), the Group VIA calcium-independent PLA_2 (GVIA $iPLA_2$) and two secreted $sPLA_2$s (GIIA and GV $sPLA_2$). The power of these assays lies in their use in determining the activity of each of these enzymes in a biological sample.

In the decade following the research that contributed to the manuscript's preparation, major advances have occurred in the PLA_2 field. Two new paralogs of the GIVA $cPLA_2$ were

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