Neuroscience Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Identification of novel phosphatidic acid-binding proteins in the rat brain

ChiHu Park[a,b,1], Du-Seock Kang[c,1], Geon-Hoon Shin[b], Jeongkon Seo[c,d], Hyein Kim[b,e], Pann-Ghill Suh[c], Chang-Dae Bae[a], Joo-Ho Shin[b,e,*]

[a] Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon, Republic of Korea
[b] Mass Spectrometry, Research Core Facility, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon, Republic of Korea
[c] School of Life Sciences, Ulsan National Institute of Science and Technology, Ulsan, Republic of Korea
[d] UNIST Central Research Facility, Ulsan National Institute of Science and Technology, Ulsan, Republic of Korea
[e] Division of Pharmacology, Department of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University, School of Medicine, Suwon, Republic of Korea

HIGHLIGHTS

- The search for phosphatidic acid (PA)-binding proteins is required for understanding PA biology.
- A lipid–affinity purification along with mass spectrometry identified 24 novel PA-binding proteins in the rat brain.
- A lipid–protein overlay assay revealed that MARCKS, GDI1, PACSIN1, and DPYSL2 are strong PA interactors.
- This list of PA-binding proteins might be helpful for deciphering the functional effects of PA in the brain.

ARTICLE INFO

Article history:
Received 5 December 2014
Received in revised form 27 March 2015
Accepted 6 April 2015
Available online xxx

Keywords:
Phosphatidic acid
Lipid–protein interaction
LC–MS/MS
Brain

ABSTRACT

Phosphatidic acid (PA) is an abundant negatively-charged phospholipid and has long been considered to be an important signaling molecule in diverse cellular events. Thus, the identification of proteins that specifically interact with PA is of considerable interest to understand the regulatory roles of PA. Herein, lipid–affinity purification and mass spectrometric analysis reveals 43 proteins, 19 known and 24 novel, as PA-binding proteins. A lipid–protein overlay assay confirmed that GDI1, PACSIN1, and DPYSL2 interact with not only with PA but also with other phospholipids. These results might be helpful for deciphering the functional effect of PA in the brain.

1. Introduction

Phosphatidic acid (PA) is a precursor in the biosynthesis of triacylglycerols and phospholipids [1], and is a lipid second messenger in mitogenic signaling pathways that has been proposed to play important roles in a wide variety of physiological events. PA normally accounts for a small percentage of the total phospholipid pool, and is very transient as it is a target for lipid phosphate phosphatases in phospholipid synthesis [2–4]. PA can be formed by phospholipase D (PLD) and diacyl glycerol (DAG) kinases. PLD can hydrolyze the phosphodiester bond of phosphatidylcholine (PC) to produce PA and soluble choline. This process has been shown to be crucial for neurite outgrowth since neuronal maturation was significantly delayed in PLD1 knockout neurons [5–6]. In addition, the phosphorylation of DAG by DAG kinase can generate PA, providing a link between lipid metabolism and subcellular signaling. Indeed, the genetic translocation of DAG kinase delta (DGKd) at 46,X,*t*(X;2)(p11.2;q37) was found in in patients with seizures, congenital capillary abnormalities, mild hypotonia, and

Abbreviations: STRAP, software tool for researching annotations of proteins; GO, gene ontology; MARCKS, myristoylated alanine-rich C-kinase substrate; GDI1, GDP dissociation inhibitors; PACSIN1, protein kinase C and casein kinase substrate in neurons1; DPYSL2, dihydropyrimidinase-like 2; GT, triglyceride; DAG, diacylglycerol; PA, phosphoatidic acid; PS, phosphatidylserine; PE, phosphatidylethanolamne; PC, phosphatidylcholine.

* Corresponding author at: Division of Pharmacology, Department of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon, Gyeonggi-do 440-746, Republic of Korea.
Tel.: +82 031 299 6192; fax: +82 031 299 6209.
E-mail address: jshin24@skku.edu (J.-H. Shin).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.neulet.2015.04.012
0304-3940/

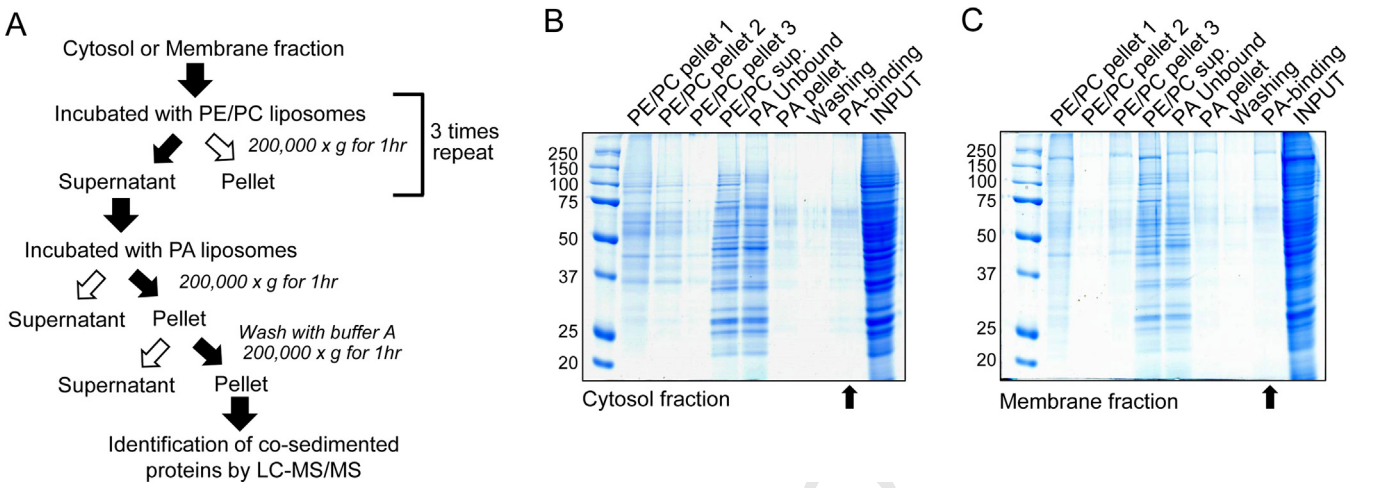

Fig. 1. Experimental scheme for the purification of PA-binding proteins in the rat brain (A). The samples were incubated with neutral liposomes (1 mg/mL) composed of PE and PC (50:50 ratio). After sedimentation, the supernatants were subsequently incubated with PA liposomes (1 mg/mL). The resultant PA-binding complexes from the cytosol (B) and the membrane fractions (C) of the rat brain were subjected to SDS-PAGE and stained with coomassie brilliant blue. PA-binding fraction was extracted from PA pellet after extensive washing step. Sup supernatant; PE, phosphatidylethanolamne; PC, phosphatidylcholine. Arrows indicate the fraction used for mass spectrometry.

mental retardation [7], suggesting that PA production is closely related with neuronal functionality and brain development.

At the molecular level, PA regulates cell polarity through interacting with various proteins such as GTPases, kinases, phosphatases, and transcription factors to modulate either catalytic activity or membrane associations [8–12]. Generally, PA-interacting proteins are involved in the regulation of actin dynamics, membrane trafficking, and neuronal development. Thus,

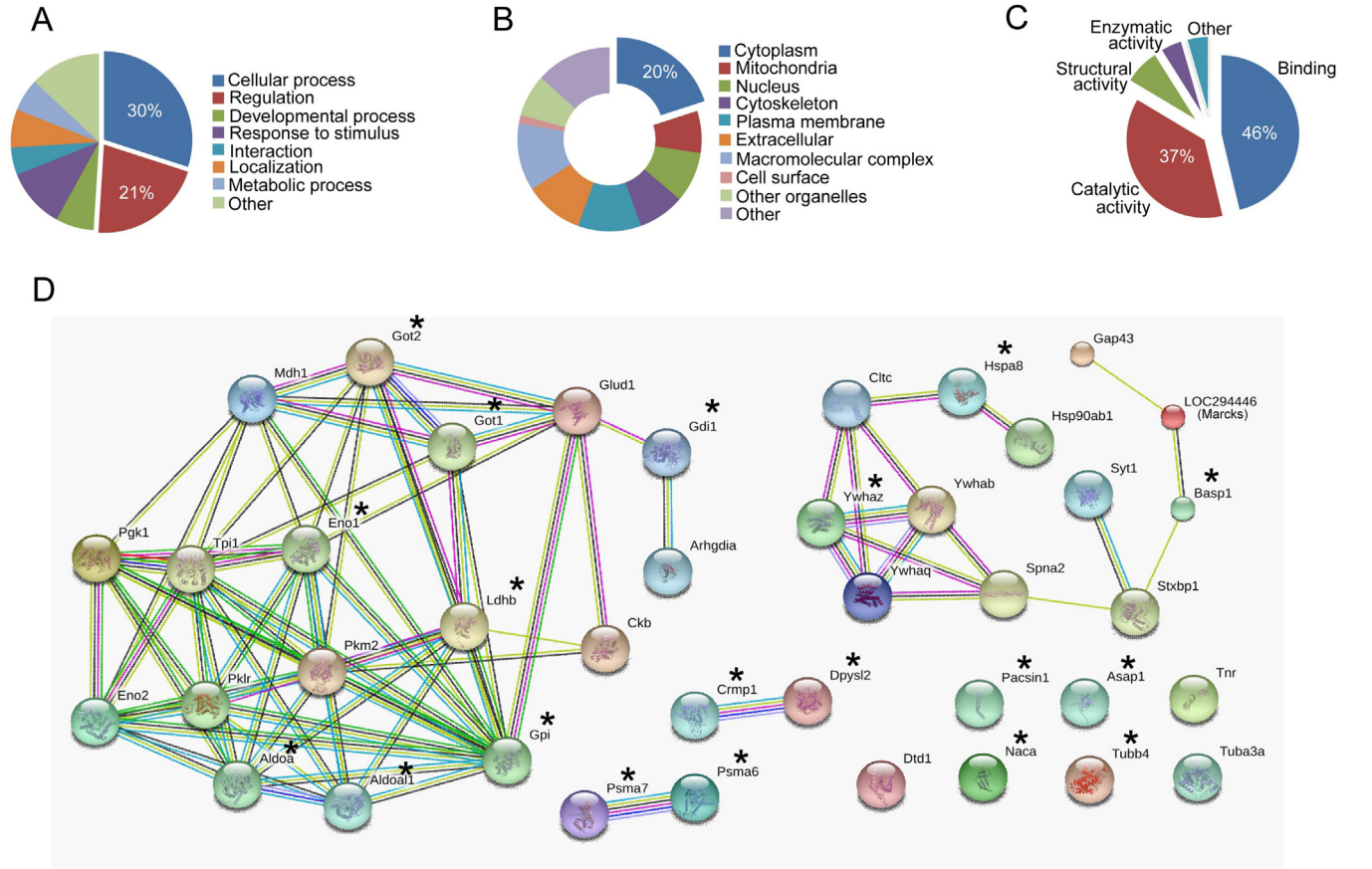

Fig. 2. A classification of commonly identified PA-binding proteins in the rat brain. STRAP software was used to annotate and visualize proteins based on gene ontology in terms of biological process (A), cellular component (B), and molecular function (C). Pie charts represented a percentage value of the subcategories. (D) The protein functional association network of the PA-binding proteins by STRING software. An edge was drawn with different colored lines representing the existence of different types of evidence. A red line, fusion; a green line, neighborhood; a blue line, co-occurrence; purple line, experimental; a yellow line, text-mining; a light blue line, database, and a block line, coexpression. Asterisks indicate PA-binding proteins that were newly identified in this study (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Please cite this article in press as: C. Park, et al., Identification of novel phosphatidic acid-binding proteins in the rat brain, Neurosci. Lett. (2015), http://dx.doi.org/10.1016/j.neulet.2015.04.012