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Phosphatidylserine-stimulated production of *N*-acyl-phosphatidylethanolamines by Ca^{2+}-dependent *N*-acyltransferase

Zahir Hussain[a], Toru Uyama[a], Katsuhisa Kawai[b], Smriti Sultana Binte Mustafiz[a], Kazuhito Tsuboi[a,c], Nobukazu Araki[b], Natsuo Ueda[a,*]

[a] Department of Biochemistry, Kagawa University School of Medicine, 1750-1 Ikenobe, Miki, Kagawa 761-0793, Japan
[b] Department of Histology and Cell Biology, Kagawa University School of Medicine, 1750-1 Ikenobe, Miki, Kagawa 761-0793, Japan
[c] Department of Pharmacology, Kawasaki Medical School, 577 Matsushima, Kurashiki, Okayama 701-0192, Japan

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ABSTRACT

N-acyl-phosphatidylethanolamine (NAPE) is known to be a precursor for various bioactive *N*-acylethanolamines including the endocannabinoid anandamide. NAPE is produced in mammals through the transfer of an acyl chain from certain glycerophospholipids to phosphatidylethanolamine (PE) by Ca^{2+}-dependent or -independent *N*-acyltransferases. The ε isoform of mouse cytosolic phospholipase A_2 (cPLA$_2$ε) was recently identified as a Ca^{2+}-dependent *N*-acyltransferase (Ca-NAT). In the present study, we first showed that two isoforms of human cPLA$_2$ε function as Ca-NAT. We next purified both mouse recombinant cPLA$_2$ε and its two human orthologues to examine their catalytic properties. The enzyme absolutely required Ca^{2+} for its activity and the activity was enhanced by phosphatidylserine (PS). PS enhanced the activity 25-fold in the presence of 1 mM $CaCl_2$ and lowered the EC_{50} value of Ca^{2+} > 8-fold. Using a PS probe, we showed that cPLA$_2$ε largely co-localizes with PS in plasma membrane and organelles involved in the endocytic pathway, further supporting the interaction of cPLA$_2$ε with PS in living cells. Finally, we found that the Ca^{2+}-ionophore ionomycin increased [^{14}C]NAPE levels > 10-fold in [^{14}C]ethanolamine-labeled cPLA$_2$ε-expressing cells while phospholipase A/acyltransferase-1, acting as a Ca^{2+}-independent *N*-acyltransferase, was insensitive to ionomycin for full activity. In conclusion, PS potently stimulated the Ca^{2+}-dependent activity and human cPLA$_2$ε isoforms also functioned as Ca-NAT.

1. Introduction

N-acyl-phosphatidylethanolamine (NAPE) represents a rare class of membrane glycerophospholipids with the third fatty acyl chain bound to the amino group of the ethanolamine moiety, and is produced in mammals through the transfer of an acyl chain from glycerophospholipids, such as phosphatidylcholine (PC), to phosphatidylethanolamine (PE) by enzymes called *N*-acyltransferase [1] (Fig. 1A). In addition to having a membrane stabilizing effect [2,3], NAPE is well known for being a precursor for various bioactive *N*-acylethanolamines (NAEs). For example, *N*-arachidonoyl-PE, *N*-palmitoyl-PE and *N*-oleoyl-PE serve as precursors for the endocannabinoid arachidonoylethanolamide (anandamide) [4], anti-inflammatory palmitoylethanolamide [5] and appetite-suppressing oleoylethanolamide [6], respectively. Therefore, *N*-acyltransferase attracts much attention as the first step of *N*-acylethanolamine biosynthesis.

A series of our studies revealed that the phospholipase A/acyltransferase (PLAAT) family proteins have an *N*-acyltransferase activity, which is not stimulated by Ca^{2+}. We also showed that the proteins of this family can abstract an acyl chain from both *sn*-1 and -2 positions of the glycerol backbone of the acyl donor, making these proteins much distinct from the long known, but molecularly uncharacterized Ca^{2+}-dependent *N*-acyltransferase (Ca-NAT) with *sn*-1 position-selectivity [1].

Recently, Ogura et al. identified Ca-NAT of mice as the ε isoform of cytosolic phospholipase A_2 (cPLA$_2$) (NCBI GenBank accession number, NM_177845) [7]. They characterized the enzyme with a crude preparation of HEK293T cells overexpressing recombinant cPLA$_2$ε and showed that the enzyme Ca^{2+}-dependently forms NAPE in the presence of PC (an acyl donor) and PE (an acyl acceptor). Moreover, the stimulation of cPLA$_2$ε-expressing cells by the Ca^{2+}-ionophore ionomycin significantly ($P < 0.01$) enhanced intracellular NAPE levels. However, since the recombinant enzyme was studied in crude preparations, it appeared to be difficult to find a possible endogenous factor(s) which alters the stimulatory effect of Ca^{2+} on this enzyme. Moreover, human orthologues of cPLA$_2$ε have not been characterized as Ca-NAT.

* Corresponding author.
E-mail address: nueda@med.kagawa-u.ac.jp (N. Ueda).

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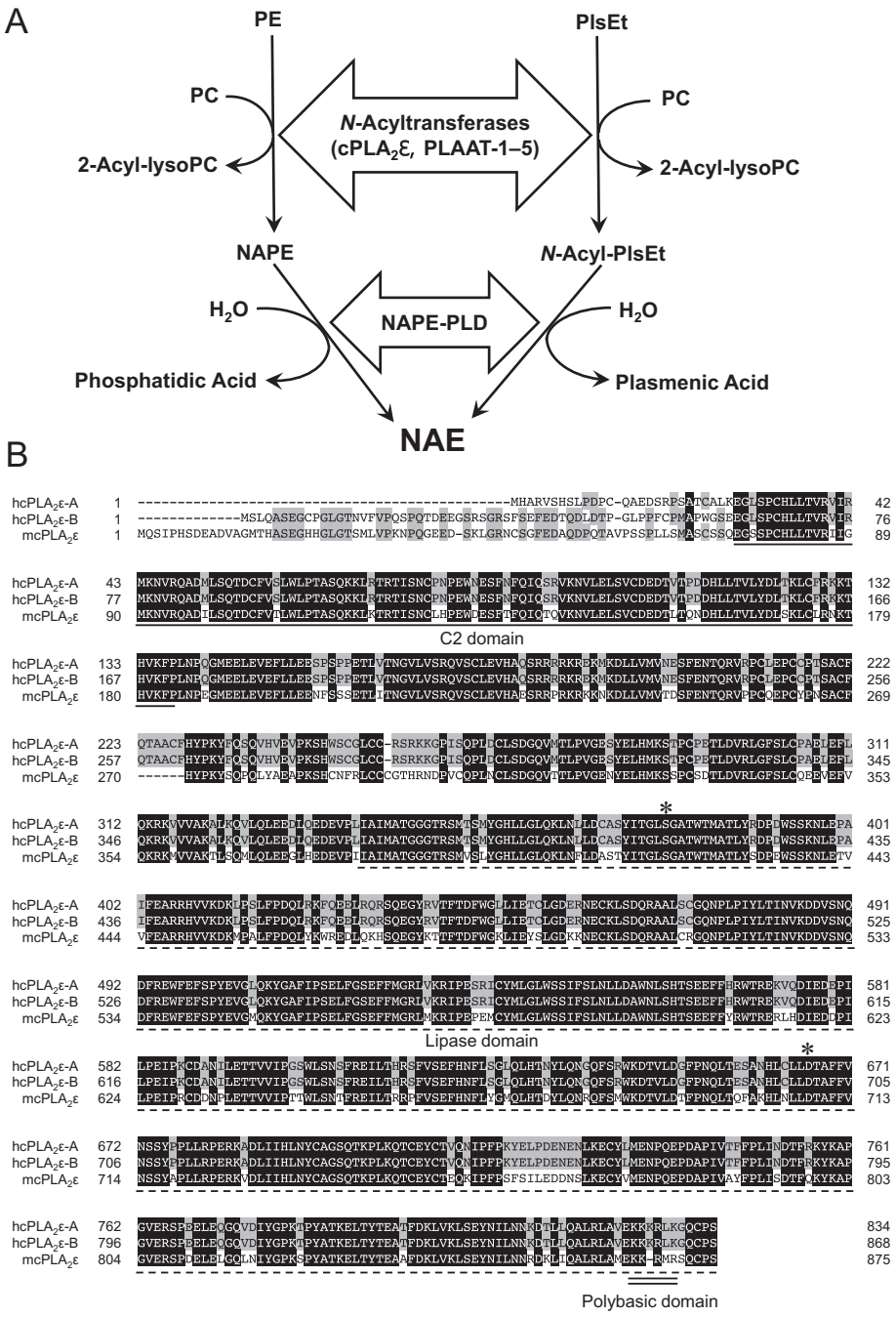

Fig. 1. The outline of NAE-biosynthetic pathway and alignment of the amino acid sequences of cPLA$_2\varepsilon$. (A) A schematic pathway for the NAE biosynthesis is shown. NAPE-PLD-independent, multi-step pathways are omitted. (B) The amino acid sequences of human and mouse cPLA$_2\varepsilon$ proteins are aligned. Two isoforms of human cPLA$_2\varepsilon$ (XM_011521237 and NM_001206670) are tentatively designated as A and B, respectively. Closed and shaded boxes indicate identity in all three and any two sequences, respectively. N-terminal C2 domain, lipase domain [17] and C-terminal polybasic domain [14] are indicated by single, dashed, and double lines, respectively. Asterisks indicate the Ser/Asp catalytic dyad. h, human; m, mouse.

In the present study, we functionally expressed two isoforms of human cPLA$_2\varepsilon$ (XM_011521237 and NM_001206670) and characterized them as Ca-NAT. We also purified recombinant mouse and human cPLA$_2\varepsilon$s and revealed the role of phosphatidylserine (PS) as an activator. Finally, we compared cPLA$_2\varepsilon$ with PLAAT-1, acting as a Ca^{2+}-independent *N*-acyltransferase, in terms of NAPE generation in the Ca^{2+}-ionophore ionomycin-treated cells. These results demonstrate that not only mouse but also human cPLA$_2\varepsilon$ functions as NAPE-generating *N*-acyltransferase, the activity of which is regulated by the combination of Ca^{2+} and PS.

2. Materials and methods

2.1. Materials

1,2-[1′-^{14}C]dipalmitoyl-PC was purchased from PerkinElmer Life Science (Boston, MA, USA). [1,2-^{14}C]ethanolamine HCl was from Moravek Biochemicals (Brea, CA, USA). PS (1,2-di-(9*Z*-octadecenoyl)-*sn*-glycero-3-phospho-L-serine) and plasmenylethanolamine (PlsEt) (1-(1*Z*-octadecenyl)-2-oleoyl-*sn*-glycero-3-phospho-ethanolamine) were from Avanti Polar Lipids (Alabaster, AL, USA). Horseradish peroxidase-linked anti-rabbit IgG antibody was from GE Healthcare (Piscataway, NJ, USA). 1,2-Dipalmitoyl-PC, 1,2-dioleoyl-PE, mouse anti-FLAG