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様式１０２

学位論文の内容の要旨

専　攻	分子情報制御医学	部　門	分子細胞医学
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論文題目	The role of intracellular anionic phospholipids in the production of *N*-acyl-phosphatidylethanolamines by cytosolic phospholipase $A_2\varepsilon$		

Introduction

N-Acylethanolamines (NAEs) represent a class of lipid mediators and refer to ethanolamides of various fatty acids with different chain length and saturation. NAEs exert various biological functions through the interaction with their corresponding receptors. For example, arachidonoylethanolamide, also known as anandamide, has attracted attention as an endogenous agonist for cannabinoid receptor CB1 and transient receptor potential vanilloid 1. NAEs are enzymatically biosynthesized from their precursors *N*-acylphosphatidylethanolamines (NAPEs). Generation of NAPEs is catalyzed by the rate-limiting enzyme, *N*-acyltransferase, which transfers an acyl chain of glycerophospholipid to the amino group of phosphatidylethanolamine (PE). In 2016, the ε isoform of cytosolic phospholipase A_2 ($cPLA_2\varepsilon$, also called PLA2G4E) was identified as Ca^{2+}-dependent *N*-acyltransferase. We recently showed by using the purified recombinant $cPLA_2\varepsilon$ that the activity is remarkably stimulated by an anionic phospholipid, phosphatidylserine (PS), *in vitro*. In this study, I investigated whether or not endogenous PS regulates the function of human $cPLA_2\varepsilon$ in living cells. I also examined the effects of other anionic phospholipids.

Methods

The expression of two endogenous PS-synthesizing enzymes (PSSs) in $cPLA_2\varepsilon$-overexpressing HEK293 cells was suppressed by their siRNAs. The *N*-acyltransferase activity of $cPLA_2\varepsilon$ was estimated by quantifying [^{14}C]NAPE produced from [^{14}C]phosphatidylcholine and non-radioactive PE as acyl donor and acyl acceptor, respectively. The localization of enhanced green fluorescent protein (EGFP)-fused $cPLA_2\varepsilon$ in HEK293 cells was observed by confocal laser microscopy. Liposomes containing various phospholipids were prepared by sonication and used for co-sedimentation assay and enzyme assay.

Results

In cPLA$_2$ε-expressing HEK293 cells, the suppression of two PSSs caused reduction in the expression level of cPLA$_2$ε as well as its *N*-acyltransferase activity. As observed by microscopy, EGFP-cPLA$_2$ε was localized to plasma membrane and intracellular vesicles, both of which were rich in PS. In the PSS-suppressing cells, both the signals of PS marker and EGFP-cPLA$_2$ε became weak in intracellular vesicles. A point mutant S412A and deletion mutants lacking C2 domain, lipase domain or polybasic domain of cPLA$_2$ε were enzymatically inactive and showed abnormal intracellular localizations. Furthermore, when assayed in the presence of liposomes or their absence, the *N*-acyltransferase activity was stimulated not only by PS but also by other anionic phospholipids such as phosphatidic acid (PA), phosphatidylinositol (PI), and PI 4,5-bisphosphate (PIP$_2$). Liposome co-sedimentation assay revealed that PA and PIP$_2$ as well as PS significantly increased the binding rate of purified cPLA$_2$ε to liposomes in the presence of Ca^{2+}.

Discussion

The knockdown of PSSs decreased both cPLA$_2$ε expression levels and *N*-acyltransferase activity, suggesting that endogenous PS plays a certain role in the function and stability of cPLA$_2$ε. Several anionic glycerophospholipids, including PS, PA and PIP$_2$, enhanced the Ca^{2+}-dependent binding of cPLA$_2$ε to liposome membrane and stimulated its *N*-acyltransferase activity. Altogether, these results suggested that endogenous PS and other anionic phospholipids affect the Ca^{2+}-dependent localization and *N*-acyltransferase activity of cPLA$_2$ε.

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