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学位論文審査の結果の要旨

平成　２９年　１月　３１日

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論文題目	Calcium-dependent generation of *N*-acylethanolamines and lysophosphatidic acids by glycerophosphodiesterase GDE7			
学位論文の審査結果	(合格)　・　不合格　（該当するものを○で囲むこと。）			

〔要旨〕

Introduction:

N-Acylethanolamines (NAEs) are ethanolamides of long-chain fatty acids. They include anandamide (an endocannabinoid), palmitoylethanolamide (an analgesic and anti-inflammatory substance), and oleoylethanolamide (an appetite-suppressor). In animal tissues, they are synthesized from a unique phospholipid, N-acyl-phosphatidylethanolamine (NAPE), in one-step reaction by NAPE-specific phospholipase D or through multi-step pathways via N-acyl-lysophosphatidylethanolamine (lysoNAPE). LysoNAPE is hydrolyzed by lysophospholipase D (lysoPLD)-type enzyme(s) to generate NAEs. Lysophosphatidic acid (LPA), a well-known lipid mediator, is also generated as another product of this reaction.

The glycerophosphodiesterase (GDE) family is a protein family characterized by highly conserved GDE domain and has seven members (GDE1-7) in mammals. GDE4 was recently shown to have lysoNAPE-hydrolyzing lysoPLD activity to generate NAEs. In the present study, I examined GDE7, together with GDE4, whether it contributes to the NAE and LPA biosynthesis by its lysoPLD activity.

Methods:

Recombinant GDE7 and GDE4 from humans and mice were overexpressed in HEK293 cells, and the membrane fractions or whole cells were subjected to Western blotting, enzyme assay, and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The tissue distributions of mRNAs for GDE7 and GDE4 in humans and mice were examined by RT-PCR. Tissue homogenates were prepared from male C57BL/6 mice and used for enzyme assays.

Results:

Following confirmation of the expression of recombinant GDE7 and GDE4 by Western blotting, both the proteins were revealed to have lysoPLD activities hydrolyzing various lysoNAPEs with different *N*-acyl species as well as the 1-alkenyl analog, *N*-acyl-lysoplasmalogen, to generate their corresponding NAEs and LPAs. GDE7 also hydrolyzed lysophosphatidylcholine to produce LPA. The activity of GDE7 was stimulated by micromolar concentrations of Ca^{2+}, but not by 2 mM Mg^{2+}. In contrast, GDE4 activity was increased by 2 mM Mg^{2+}, but not by 2 mM Ca^{2+}. LC-MS/MS analyses showed that most of NAE and LPA species were increased in GDE7-overexpressing HEK293 cells. Although GDE7 and GDE4 mRNAs were widely distributed in various tissues of humans and mice, GDE7 mRNA was abundant in kidney and GDE4 was highly expressed in brain and testis. In consistence with high expression levels of GDE7 in mouse kidney, EGTA, a calcium-specific chelator, decreased NAE-forming activity of mouse kidney homogenate.

Discussion:

I showed for the first time that GDE7 has a lysoPLD activity toward lysoNAPE. The activation of GDE7 by submicromolar concentration of Ca^{2+} suggested that GDE7 could produce NAE and LPA in response to physiological stimuli to increase intracellular Ca^{2+} levels. The decrease in the lysoPLD activity of mouse kidney homogenate by EGTA suggested that the lysoPLD activity in this organ may be at least partially attributed to GDE7.

These results suggested that GDE7 is a novel Ca^{2+}-dependent lysoPLD, which is involved in the biosynthesis of NAEs and LPAs.

本論文に関する学位論文審査委員会は平成 29 年 1 月 20 日に行われ、以下の質疑応答が行われた。

1. Could you predict the active site residues and topology of GDE7?
The active site residues of GDE7 are predicted to exist in the GDE domain between two putative transmembrane domains. The active site may be localized in the cytoplasmic site of endoplasmic reticulum (ER) membrane and activated by micromolar concentration of calcium in response to cellular stimuli. Alternatively, the active site could be localized in the extracellular side of plasma membrane and constitutively activated by extracellular calcium.

2. Do you have any experimental plan to examine membrane topology of GDE7?
In order to reveal membrane protein topology of GDE7 in ER, I will employ the spatially confined actions of proteases on the degradation of GDE7 tagged on either or both of the N/C-termini, followed by staining with anti-tag antibody. In this experiment I will use selective permeabilization of the plasma membrane by the detergent digitonin, while this treatment will leave intracellular organelles intact.

3. Can you explain the physiological role of GDE7?
GDE7 functions as a lysoPLD type enzyme to produce bioactive NAEs and LPAs. So these bioactive lipid mediators can bind to various receptors to initiate cellular responses. KO mice may reveal the answer about the physiological role of GDE7 at the animal level.

4. What determines the calcium-dependency of GDE7 and magnesium-dependency of GDE4?
There is no known calcium binding motif or magnesium binding motif in amino acid sequences of GDE7 and GDE4, respectively. The analyses of GDE7/GDE4 chimera and point mutants will be helpful for identifying the regions determining the dependency on calcium and magnesium. Large-scale preparation and purification of GDE7 and crystallography analysis will also give valuable information for this question.

5. Why the activity of GDE4 is lower than GDE7 when expressed in HEK293 cells?
The lower activity of GDE4 may result from its lower expression levels under the experimental conditions which I used. In addition, the possibility of the presence of better substrate for GDE4 cannot be ruled out.

本研究は脂質メディエーターである *N*-アシルエタノールエタノールの生合成における GDE7 の役割を初めて解析した研究であり、結果に対する十分な考察もなされている。本研究で得られた成果は本脂質メディエーターの代謝の包括的理解に貢献し、学術的意義も大きい。審査委員会において、申請者はいずれの質問にも的確に回答し、博士（医学）の学位授与に値する見識と能力を有することが認められた。よって、審査委員会は、本論文が博士（医学）の学位論文に十分値するものと判定した。

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（備考）要旨は，１，５００字以内にまとめてください。