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Title	Study on n-3 polyunsaturated fatty acid-binding phosphatidylglycerol : enzymatic synthesis and biofunctional evaluation [an abstract of dissertation and a summary of dissertation review]
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Citation	北海道大学. 博士(水産科学) 甲第13885号
Issue Date	2020-03-25
Doc URL	http://hdl.handle.net/2115/77878
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Type	theses (doctoral - abstract and summary of review)
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	Liping_Chen_abstract.pdf (論文内容の要旨)

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学位論文内容の要旨

博士の専攻分野の名称：博士（水産科学）　　氏名：陳 莉萍

学 位 論 文 題 目

Study on n-3 polyunsaturated fatty acid-binding phosphatidylglycerol: enzymatic synthesis and biofunctional evaluation

（n−3 系高度不飽和脂肪酸結合ホスファチジルグリセロールの酵素的合成と生体機能評価に関する研究）

Phosphatidylglycerol (PG) is a highly functional phospholipid (PL) with glycerol as a negatively charged polar head group. PG has serval physiological functions and shows excellent liposome forming activity based on amphiphilic properties. Though it is a ubiquitous PL in the biological membranes of many organisms, naturally occurring PG binding n-3 polyunsaturated fatty acids (n-3 PUFA-PG) typically as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is low, resulting in a scarcity of industrial bioresources of n-3 PUFA-PG. In **Chapter 1**, preparation of n-3 PUFA-PG was conducted from salmon roe lipids and glycerol *via* phospholipase D (PLD)-mediated transphosphatidylation. In this study, to establish industrial reaction system for n-3 PUFA-PG synthesis, salmon roe total lipid (n-3 PUFA-TL) and salmon roe phospholipid (n-3 PUFA-PL) as well as phosphatidylcholine purified salmon roe (n-3 PUFA-PC) were used as substrates and investigated the optimum reaction conditions. n-3 PUFA-PG yields obtained from n-3 PUFA-TL n-3 PUFA-PL were higher than that obtained from n-3 PUFA-PC in aqueous system with the exemption of organic solvent. Following a 24-h reaction with 0.75 U PLD, n-3 PUFA-TL and n-3 PUFA-PL yielded up to 96.4 mol% and 96.7 mol% n-3 PUFA-PG, respectively, in the aqueous reaction system.

In **Chapter 2**, hydrolysis of n-3 PUFA-PG by pancreatic enzymes was investigated to clarify nutritional property of n-3 PUFA-PG. Fatty acids which largely comprised of n-3 PUFAs at *sn*-2 position of n-3 PUFA-PG were rapidly liberated *via* hydrolysis by phospholipase A_2 (PLA_2) than from n-3 PUFA-PC and soybean PC in *in-vitro* digestion model. Fatty acids at the *sn*-2 position of n-3 PUFA-PG were firstly hydrolyzed by pancreatic PLA_2 during a 6-h reaction, whereas fatty acids of n-3 PUFA-PC were partially unhydrolyzed even after a 24-h reaction. Those results suggest that n-3 PUFA-PG converted from salmon lipids by PLD is a functional PL with high bioavailability of n-3 PUFAs.

Chronic inflammation has been clarified to represent a triggering factor in the origin of the metabolic syndrome. Thus, prevention and resolution of inflammation is of great importance for the maintenance of body health. In **Chapter 3**, anti-inflammatory effect of n-3 PUFA-PG was estimated upon lipopolysaccharide (LPS)-stimulated macrophages RAW264.7. It is noteworthy

that anti-inflammatory activity of n-3 PUFA-PG was strong compared to n-3 PUFA-PC with slight variation in fatty acid composition. n-3 PUFA-PG upregulated mRNA expression of antioxidative enzymes heme oxygenase-1 (HO-1) and quinone oxireductase-1 (NQO-1) prior to LPS stimulation. The obtained data show that compared to n-3 PUFA-PC, n-3 PUFA-PG presented a remarkably inhibition of inflammation with alleviation of the transcriptional levels of inflammatory cytokines interleukin-6 (IL-6), interleukin-1β (IL-1β), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), monocyte chemotactic protein-1 (MCP-1). Furthermore, production of the nitric oxide (NO) production, IL-6 and IL-1β were also inhibited in RAW264.7 cells treated with n-3 PUFA-PG. Furthermore, in RAW264.7 cells treated with n-3 PUFA-PG, n-3 PUFAs were incorporated predominately compensated by decrease of oleate (18:1n-9), accompanied with a lowered n-6 to n-3 PUFA ratio, and mainly accreted in esterified form of cellular membrane phosphatidylcholine (PE). Anti-inflammatory activity exhibited by n-3 PUFA-PG may be associated with the elevated alteration of cellular n-3 PUFAs proportional levels. Taken together, it can be concluded that anti-inflammation property of n-3 PUFAs may depend on their chemical carriers, n-3 PUFA-PG exerts stronger ameliorative potency on LPS-induced inflammation in RAW264.7 cells.

In **Chapter 4**, the health benefits and tissue accretion of dietary n-3 PUFA-PG were further investigated in diabetic/obese KK-A^y mice. After a feeding duration over 30 days, n-3 PUFA-PG significantly reduced the total and non-HDL cholesterols in the serum of diabetic/obese KK-A^y mice. In the mice fed n-3 PUFA-PG, but not n-3 PUFA-triglyceride (n-3 PUFA-TAG), hepatic lipid content was markedly alleviated depending on the neutral lipid reduction compared with that of the SoyPC-fed mice. Further, the n-3 PUFA-PG diet increased EPA, DHA and reduced arachidonic acid in the small intestine, liver, perirenal white adipose tissue, and brain, and the ratio of n-6 to n-3 PUFA in those tissues became lower compared to the SoyPC-fed mice. Especially, the DHA level was more significantly elevated in the brains of n-3 PUFA-PG-fed mice compared to the SoyPC-fed mice, whereas n-3 PUFA-TAG did not significantly alter DHA in the brain. The present results indicate that n-3 PUFA-PG is a functional lipid for reducing serum and liver lipids and is able to supply n-3 PUFAs to KK-A^y mice.

In conclusion, n-3 PUFA-TL, n-3 PUFA-PL and n-3 PUFA-PC from salmon roe were useful substrates for the synthesis of n-3 PUFA-PG by PLD-mediated transphosphatidylation. n-3 PUFAs were predominantly esterified to *sn*-2 position of n-3 PUFA-PG. In *in-vitro* digestion model, n-3 PUFA-PG showed high affinity with PLA_2 and rapid liberation was observed. Compared to n-3 PUFA-PC, n-3 PUFA-PG with similar fatty acid profiles exerted potent anti-inflammatory effect and show the high bioavailability of n-3 PUFAs. In *in-vivo* experiment of diabetic/obesity mice KK-A^y, n-3 PUFA-PG is a functional bioavailable lipid source for n-3 PUFAs and is at least the same or better than the n-3 PUFA-TAG. The present results indicate that synthesized n-3 PUFA-PG is a high potential lipid with health benefits.