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博士の専攻分野の名称	博士（理学）
学位記号番号	博理工甲第 916 号
学位授与年月日	平成 25 年 9 月 20 日
学位授与の条件	学位規則第 4 条第 1 項該当
学位論文題目	IDENTIFICATION AND CHARACTERIZATION OF PROTEINS THAT BIND SPECIFIC LIPID DOMAINS （特異的脂質ドメインに結合するタンパク質の同定と解析）
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論文の要約

Background:

Membrane rafts are transient and unstable membrane microdomains that are enriched in sphingolipids, cholesterol, and specific proteins. These microdomains can be stabilized on binding ligand molecules. They have been shown to exist as nanoscale clusters of various sizes. The membrane rafts have been implicated in a huge range of cellular processes, and are therefore under intense study. Some examples of where rafts are thought to play a role includes, T-cell activation and the T-cell synapse, B-cell activation, focal adhesions and cell migration, the life cycle of the influenza and HIV viruses, hormone signaling and membrane trafficking in polarized epithelial cells. The existence and function of these membrane rafts/domains are often demonstrated by indirect biochemical methods such as using nonionic detergent at low temperature or cholesterol depletion by methyl-β-cyclodextrin. However, some problems with these techniques have been pointed out. Recent development of proteins and peptide probes that bind specific lipids, together with advanced optical and electron microscopy techniques have opened up the studies to clarify the distribution and dynamics of the lipids. Although useful, one has to carefully use these protein probes to avoid the toxicity to cells and the rearrangement of lipids during observation.

Aim of this study:

The aim of my study was to isolate a non-toxic protein binding to specific lipid domains from mushroom extracts and further characterize the protein.

Summary of results:

The present study reveals that the proteins belonging to aegerolysin family are new lipid raft probes for both mammalian and insect cells.

In my study two sphingomyelin (SM) probes, lysenin and equinatoxin II were used to know the subcellular

distribution of SM, and the behavior of these two proteins with respect to it. The SM pool distribution in the plasma membrane of COS-1 cells was studied, followed by examining the intracellular distribution of SM and SM synthases involved in the generation of the intracellular SM pools.

Further screening for sterol and sphingolipid enriched membrane domain binding protein was carried out using mushroom as protein source, with various combinations of artificial liposome mixture. In the process of screening three proteins were isolated. One from *H.marmoreus* (bunashimeji mushroom) binding specifically to GM1. The other two proteins were from *P.eryngii* (eryngii mushroom), were one bound to GM1/cholesterol(Chol) and the other bound to SM/Chol.

These proteins were identified by peptide sequencing and mass spectrometry analysis. But the various approaches followed to purify GM1 and GM1/Chol binding protein failed. Whereas, SM/Chol binding protein was successfully purified both in natural and recombinant form.

This newly isolated protein was identified as an ortholog of pleurotolysinA. Therefore naming the protein as pleurotolysinA2 (PlyA2). NCBI database search revealed that PlyA2 belongs to aegerolysin family and exhibits a high homology to other family members, erylysinA (EryA) and ostreolysin (Oly) (80 % identity). The recombinant PlyA2 conjugated to enhanced green fluorescent (EGFP) was expressed in *E.coli* and the fluorescent protein was used to characterize its lipid specificity in model and cellular membranes. Further the binding affinity of aegerolysin family proteins to SM substituent, ceramide phosphorylethanolamine (CPE) which is found in insects like *Drosophila* and parasitic pathogens like *Trypanosomes* was carried out.

Conclusion:

SM probes, lysenin and equinatoxin II revealed different SM pools with distinct affinity for both the toxins. The study revealed that SMS2 and SMS1 enzymes are responsible for the synthesis of SM in the plasma membrane and endomembrane, respectively, in COS-1 cells. The use of these two SM-binding probes may provide more insights into various SM mediated processes in different topological domains.

In the process of screening for protein binding to sphingolipid/sterol enriched domain, a SM/Chol binding protein was isolated which was named as pleurotolysinA2. PlyA2-EGFP was successfully characterized via *in vitro* and *in vivo* assays. PlyA2-EGFP *in vitro* studies revealed the protein binding is based on direct protein-lipid headgroup interaction and not due to the liquid ordered state of the membrane. While *in vivo* studies showed that PlyA2-EGFP selectively binds to a SM/Chol complex on the cell surface, rendering it a suitable probe to label SM/Chol rich membrane domains. The behavior of PlyA2-EGFP with other recombinant aegerolysin family proteins was compared and only PlyA2-EGFP retained its SM/Chol binding activity.

This study issued a question of the lipid target of these aegerolysin family proteins, since they have 80% identical amino acids. Previous reports have revealed about the substitution of SM into ceramide phosphorylethanolamine (CPE) in insects like *Drosophila*, and parasitic pathogens like *Trypanosomes, Leishmania*. Henceforth promising an interesting study on the binding of aegerolysin family proteins to CPE-rich or CPE/Chol-rich domains. The *in vitro* and *in vivo* assays of the aegerolysin family proteins acknowledged, PlyA2 to be able to bind CPE/Chol- and CPE-enriched domains whereas, EryA and Oly bound to CPE/Chol-enriched domains.

In essence, PlyA2-EGFP is a non-toxic lipid binding protein capable to selectively associate either with SM/Chol-rich membrane domains or CPE-rich membrane domains, alongside other aegerolysin family proteins EryA and Oly capable of selectively associating with CPE/Chol-rich membrane domains. In conclusion, PlyA2-EGFP is a complementary addition to the lipid probe toolkit to study lipid distribution and dynamics *in situ*.