学 位 論 文 審 査 の 要 旨

博士の専攻分野の名称　博士（生命科学）　氏　名　Mahadeva Swamy M.M

審査担当者	主査	教　授　門出　健次
	副査	教　授　出村　　誠
	副査	教　授　西村　紳一郎
	副査	教　授　木原　章雄（薬学研究院）
	副査	助　教　村井　勇太

学 位 論 文 題 名

Discovery of Naturally Occurring Sphingomyelin Synthase Inhibitors: Structural Activity Relationship Validation and Inspiring Sphingo-mimic Studies

（スフィンゴ脂質代謝を制御する天然有機化合物の探索とその機能解明に関する研究）

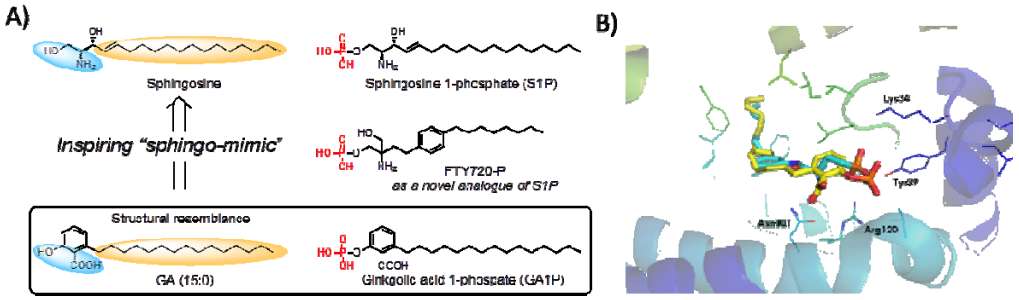
Sphingolipids are class of lipids having a backbone of sphingoid base with amide linked fatty acids and having different polar head groups. They are first discovered from the brain extract in 1870s. Sphingolipids are named after the mythological Sphinx because of their enigmatic nature. They are known to protect cell surface by forming plasma membrane lipid bilayer. These sphingolipids play an important role in signal transmission and cell recognition, involved in many cellular processes such as apoptosis, senescence, differentiation, autophagy etc. Among variety of sphingolipids, ceramide, sphingomyelin and sphingosine 1-phosphate (S1P) acquired major attention in the field of drug discovery. Shingomyelin synthase (SMS) is an enzyme to biosynthesize sphingomyelin that regulates membrane fluidity and microdomain structure. The SMS isoform, SMS1 play a crucial role to maintain cell homeostasis but the plasma membrane enzyme, SMS2 has significant role in mediating metabolic syndrome. Recent report suggests that the deficiency SMS2 attenuates the development of obesity, fatty liver and type 2 diabetes and involved in other metabolic disorders such as insulin resistance and atherosclerosis. SMS2 activity has also been involved in Alzheimer's disease and tumorigenesis, thus SMS2 could serve as a promising therapeutic target for these diseases.

Initial goal of our study was to identify potent and selective SMS2 inhibitors from the natural source thus; we screened a library of 650 plants from Hokkaido against SMS. As a result, ginkgolic acid (GA) from *Ginkgo biloba* has been identified as a first natural, potent SMS inhibitor but it is not selective towards SMS2, also inhibits SMS1 with the same potency and succeeded in GA's total synthesis by novel methodology. To identify selective SMS2 inhibitor from GA, structural activity relationship (SAR) study was applied. As a result, carboxylic acid and the long hydrophobic chain of GA are very essential for SMS inhibition. Surprisingly, GA structure resembles the structure of sphingosine made an assumption that GA might be behaving as a substrate. To prove sphingosine like behavior of GA we applied chemical method to synthesize few sphingosine mimics and several ceramide mimics were synthesized. Chemical method, suggested us GA might be "sphingosine mimic" and also gave few selective SMS2 inhibitors, which can be used to understand the SMS2 biology in metabolic disorders and Alzheimer's disease. Due to sphngosine like behavior of GA, we named it has "sphingo-mimic". We went ahead to prove "sphingo-mimic" nature of GA by cell based assays.

Sphingosine1-phosphate (S1P), a signaling molecule for sphingosine-1-phosphate receptors ($S1P_1$-$S1P_5$) of G protein coupled receptors (GPCRs). S1P is major regulator of vascular and immune system, which binds to S1P receptors and regulates angiogenesis, vascular stability, and permeability. Inhibition of S1P receptors especially $S1P_1$ was shown to be critical for immunomodulation. FTY720 (Fingolimod), is an S1P mimic & agonist for S1P receptors and it is an FDA approved drug for the

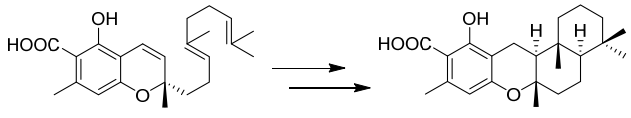
treatment of multiple sclerosis, which inspired us to synthesize a phosphate derivative of ginkgolic acid (GA1P) as S1P mimic. The agonist activity and S1P mimic nature of GA1P has been confirmed by induction of extracellular signal-regulated kinases (ERK) phosphorylation in dose dependent manner. Furthermore, the $S1P_1$ internalization upon treatment with GA1P gave strong evidence of its agonism. The selectivity of GA1P to S1P1 over other S1P receptors need to be identified and we expect that the GA1P fall in the same line as FTY720 and needs to be explored in immune response disorders.

ML5, is a reported antagonist of $S1P_1$ and sphingolipid mimic, which was used to generate crystal structure of $S1P_1$. To compare mode binding of S1P mimic GA1P with ML5, we performed *in silico* docking studies using auto-dock vina. As a result GA1P adopts similar binding pose to the bound ligand ML5, which further confirms that the GA1P is an S1P mimic. Collectively, GA is a first natural inhibitor of SMS, deep understanding of SAR study provided selective SMS2 inhibitors and GA is a first natural "sphingo-mimic".



A) Our new concept GA inspires "sphingo-mimic": Structural resemblance between GA and sphingosine. B) *In silico* docking mobel of GA1P (yellow) with bound ligand ML5 (cyan) to $S1P_1$.

On the other hand screening of medicinal plants library identified daurichromenic acid (DA) isolated from *Rhododendron dauricum* as potent SMS inhibitor. DA is a potent SMS2 inhibitor (IC_{50}- 4 µM) but failed to show selectivity towards SMS2 also inhibits SMS1 (IC_{50}- 4 µM). SAR studies of DA suggested that the carboxylic acid functional group is very essential for SMS inhibition. Lewis acid catalyzed conversion of DA undergoes cycloaddition reaction to get unnatural hongoqurcin A, which is turned out to be potent SMS inhibitor (IC_{50}- 3 µM on both SMS1 and SMS2). SMS biology in metabolic disorders and alzheime's disease need to be addressed using DA and hongoquercin A. Circular dichroism (CD) calculations of flexible natural products like DA have been difficult because of the large number of low-energy conformers and ambiguous Boltzmann distributions. Using ECD and VCD spectroscopy on DA, we demonstrated that derivatization of flexible to rigid molecule reduces the computational expenses required in identifying absolute configuration.



In conclusion, GA and DA are the first natural inhibitors of SMS, their efficiency need to be addressed in metabolic disorders and Alzheimer's disease. SAR studies of GA helped us to deeply understand the sphingosine like behaviour of GA and also to obtain few selective SMS2 inhibitors. Our assumption towards GA might be natural "sphingo-mimic" has been proved by cell based assay and by in silico docking studies.

Therefore, we acknowledge that the author is qualified to be granted the Doctorate of Life Science from Hokkaido University.