博 士 論 文 の 要 約

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学 位 論 文 題 名

Development of analytical method for monitoring sphingomyelin synthase and discovery of its natural inhibitor from *Hizikia fusiformis*
（スフィンゴミエリン合成酵素のモニタリング分析法の開発とヒジキからの天然阻害剤の発見）

Lipids are biomolecules that form the structural framework of cellular membranes, store and release energy, and function as critical mediators in numerous signaling pathways essential for physiological homeostasis. Among these, sphingolipids represent a structurally diverse and bioactive lipid class that makes cell fate decisions, including apoptosis, proliferation, differentiation, and inflammatory responses. Within the sphingolipid metabolic pathway, sphingomyelin synthase (SMS) occupies a pivotal regulatory role, catalyzing the transfer of a phosphorylcholine headgroup from phosphatidylcholine (PC) to ceramide (Cer) to yield sphingomyelin (SM) and diacylglycerol (DAG). This enzymatic reaction not only modulates the balance between SM and Cer but also influences downstream lipid-mediated signaling cascades that control membrane microdomain organization, stress responses, and metabolic homeostasis.

Dysregulation of ceramide to sphingomyelin interconversion has been increasingly associated with pathological conditions such as fatty liver disease, obesity, atherosclerosis, neurodegenerative disorders, and certain cancers. Elevated SMS activity, in particular, has been linked to lipid imbalance and proinflammatory signaling that contribute to disease progression. Despite its biological importance, research into SMS modulators especially natural inhibitors with nutritional or pharmacological potential has been limited by the lack of sensitive, robust, and physiologically relevant assay systems. The goal of this thesis was, therefore, to (1) develop a reliable and analytical platform for monitoring SMS activity, and

(2) identify naturally occurring bioactive compounds, particularly from marine sources, capable of modulating sphingomyelin metabolism. (3) objective extended the scope toward the green synthesis of bioactive nanomaterials from marine biomass, exploring their potential roles in both lipid regulation and biotechnological applications.

The thesis begins by establishing the biochemical and physiological background of sphingolipid metabolism, highlighting the interdependence between ceramide and sphingomyelin pools. Chapter 1 outlines the structural diversity of sphingolipids, their roles in maintaining membrane integrity, and their participation in cell signaling pathways. Particular attention is given to the SMS enzyme family, primarily comprising the isoforms SMS1 and SMS2, which differ in subcellular localization, regulatory mechanisms, and tissue distribution. The chapter also surveys the current knowledge on synthetic and naturally derived SMS inhibitors, summarizing their mechanisms of action and pharmacological significance. By identifying key research gaps namely, the absence of efficient cell-based analytical platforms and the underexploration of marine bioresources the chapter defines the core problem statement and sets the foundation for subsequent experimental development.

Chapter 2 presents the development of a highly specific and reproducible assay for quantifying SMS activity in living cells. The methodology integrates cell-based assay with liquid chromatography-tandem mass spectrometry (LC–MS/MS) to directly measure sphingomyelin formation from a non-fluorescent short-chain ceramide substrate (C6-ceramide). HeLa cells stably expressed with human SMS1 and SMS2 isoforms served as the model systems, ensuring physiologically relevant enzymatic environments. This analytical platform effectively bypasses the limitations of traditional fluorescence-based methods, which often suffer from high background signals and poor substrate specificity.

The developed assay was validated using ginkgolic acid (C15:1), a known natural inhibitor derived from *Ginkgo biloba*. Analysis yielded inhibitory concentrations (IC_{50}) of 5.5

μM for SMS1 and 3.6 μM for SMS2. Complementary molecular docking simulations confirmed that ginkgolic acid interacts stably within the catalytic pocket of both isoforms through hydrophobic and hydrogen bonding interactions. This dual validation established a robust biochemical computational workflow for subsequent inhibitor screening, forming a cornerstone of the thesis methodology.

Following validation of the analytical platform, Chapter 3 focuses on the bioassay-guided discovery of natural SMS inhibitors from the edible brown alga *Hizikia fusiformis* (hijiki), a marine biomass known for its diverse bioactive lipidome. Sequential extraction and chromatographic fractionation led to the isolation of cis-9-docosenoic acid (9Z-DSA) as the major inhibitory constituent. LC–MS-based assays confirmed concentration-dependent SMS inhibition, with IC_{50} values of 4.5 μM for SMS1 and 6.0 μM for SMS2, indicating strong potency comparable to established inhibitors. *In silico* docking and molecular dynamics (MD) simulations revealed stable binding of 9Z-DSA within the catalytic groove of SMS, supported by hydrophobic interactions with key active-site residues. These results not only illuminate the molecular basis of inhibition but also support the marine lipid's therapeutic relevance. The chapter proposes that naturally occurring long-chain unsaturated fatty acids such as 9Z-DSA act as physiological regulators of sphingolipid metabolism, potentially mitigating metabolic and inflammatory dysfunctions characteristic of obesity and hepatic steatosis.

In Chapter 4, the research scope expands from molecular inhibitor screening to the exploration of hijiki-derived carbon dots (H-CDs) as multifunctional nanomaterials with biological relevance. Using a one-step hydrothermal carbonization approach, the biomass of H. fusiformis was converted into ultrasmall (0.85-3 nm) carbon dots exhibiting bright blue emission ($\lambda_{em} = 407$ nm, quantum yield 8.7%) and rich surface functionalities such as hydroxyl, carboxyl, and amino groups. Structural analyses by TEM, FTIR, and XPS

confirmed their composition and surface chemistry, while optical characterization revealed excellent water solubility and colloidal stability. Interestingly, the H-CDs demonstrated significant inhibition of SMS1 and SMS2 activities *in vitro,* suggesting a novel type of nanomaterial-enzyme interaction. These findings introduced an additional dimension to the thesis: the potential for biomass-derived nanostructures to modulate lipid-metabolizing enzymes. Beyond their biocompatibility and photoluminescence, H-CDs may thus serve as both diagnostic probes and functional bioactives, merging nanotechnology with lipid biochemistry in an environmentally sustainable framework.

Chapter 5 systematically investigates the inhibitory effects of structurally diverse free fatty acids on SMS activity, employing an integrated experimental-computational approach. Among the tested compounds, pentadecanoic acid (C15:0) emerged as a particularly potent dual inhibitor of SMS1 and SMS2. Experimental assays revealed concentration-dependent inhibition consistent with the potency of known inhibitors. Docking simulations showed that C15:0 forms stable hydrophobic and van der Waals interactions within the catalytic pocket, engaging key residues critical for SMS catalysis. MD simulations further confirmed high conformational stability and minimal deviation in complex geometry, reinforcing the enzyme–ligand compatibility and dynamic persistence of binding interactions. These results suggested a mechanistic link between odd-chain fatty acids (OCFA) and the regulation of sphingolipid metabolic flux, positing C15:0 as a biologically relevant, diet-derived regulator of membrane lipid remodeling and signaling processes.

Chapter 6 extends the biochemical findings into an *in vivo* context. A dietary supplementation study using a high-fat diet-induced obesity mouse model demonstrated that C15:0 exerts broad protective effects on the heart, liver, and spleen. Compared to control groups, C15:0-fed mice exhibited attenuated body weight gain, improved lipid profiles, reduced hepatic steatosis, and normalization of membrane sphingolipid composition.

Transcriptomic and histological analyses revealed suppression of proinflammatory gene expression and enhanced expression of lipid metabolic regulators, suggesting systemic metabolic restoration. Collectively, these outcomes establish C15:0 as a nutritionally active fatty acid capable of mitigating the deleterious effects of excessive SMS activity and sphingomyelin accumulation. This evidence provides molecular justification for revisiting dietary guidelines that broadly categorize all saturated fats as detrimental, distinguishing bioactive OCFAs as beneficial lipid species with potential cardiometabolic and anti-inflammatory roles.

Through the integration of analytical, biochemical, computational, and *in vivo* studies, this thesis establishes a framework for studying sphingomyelin synthase and its modulation by natural and synthetic compounds. The developed LC–MS based cell assay overcomes major methodological challenges in enzyme activity quantification, enabling precise and reproducible analysis across isoforms. The identification of marine-derived fatty acids (9Z-DSA) and nutritionally relevant OCFAs (C15:0) as effective SMS inhibitors demonstrate the untapped potential of natural lipids as regulatory molecules. Furthermore, the incorporation of hijiki-derived carbon dots bridges biochemical research with nanomaterial science, suggesting innovative directions for lipid-targeted biofunctional materials.

Overall, the research illustrates how sphingomyelin metabolism can be modulated through marine and dietary resources. It also highlights promising translational avenues for developing nutraceutical strategies aimed at improving lipid-associated metabolic disorders. By uniting cellular biochemistry, lipidomics, molecular modeling, and material synthesis within a single framework, this work advances both scientific understanding and practical applications in marine-derived therapeutics and lipid-based health modulation.