学 位 論 文 内 容 の 要 旨

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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）

Abstract

Lipids are fundamental biomolecules that play essential roles in maintaining cellular architecture, providing energy reserves, and mediating signaling pathways essential for homeostasis. Among them, sphingolipids constitute a important class of bioactive lipids that regulate apoptosis, inflammation, and membrane organization. Sphingomyelin synthase (SMS), a key enzyme in the sphingolipid pathway, catalyzes the synthesis of sphingomyelin (SM) and diacylglycerol (DAG) from ceramide (Cer) and phosphatidylcholine (PC). Owing to its regulatory position at the intersection of sphingomyelin and ceramide metabolism, the dysregulation or overexpression of SMS activity has been implicated in multiple pathological conditions, including fatty liver disease, atherosclerosis, neurodegenerative diseases, and colon cancer. The aim of this thesis is to develop a robust analytical platform for monitoring SMS activity and to discover food-derived natural inhibitors using the bioactive brown alga *Hizikia fusiformis* as a primary source. Furthermore, to prepare the biomass-based carbon dots from Hijiki for biological applications.

Chapter 1 provides an overview of lipid and sphingolipid biology, emphasizing how SMS controls the interconversion of ceramide and sphingomyelin, thereby regulating membrane organization, apoptosis, inflammation, and disease-related signaling. It also summarizes SMS isoforms, their regulation, and current synthetic and natural inhibitors, establishing SMS as a promising therapeutic target in metabolic, neurodegenerative, and cardiovascular disorders.

Chapter 2 details the development of a sensitive, fluorescence-independent, cell-based assay integrated with liquid chromatography–tandem mass spectrometry (LC–MS/MS) for precise determination of SMS1 and SMS2 activities. HeLa cells stably expressing human SMS isoforms were utilized as a model system, and a non-fluorescent C6-ceramide substrate was employed to accurately track the enzymatic conversion to C6-sphingomyelin. This methodology overcomes the limitations of conventional fluorescence-based assays, enabling highly specific and reproducible detection of SMS activity. The assay was validated using ginkgolic acid (C15:1), a well-characterized SMS inhibitor, which exhibited half-maximal inhibitory concentrations (IC_{50}) of 5.5 μM and 3.6 μM for SMS1 and SMS2, respectively. Complementary molecular docking simulations further confirmed the stable binding of ginkgolic acid within the catalytic pocket of both isoforms, supporting the biochemical observations and reinforcing the reliability of the

developed analytical platform.

Chapter 3 investigates the identification of natural inhibitors of SMS derived from *Hizikia fusiformis*. Bioassay-guided extraction and chromatographic purification led to the isolation of cis-9-docosenoic acid (9Z-DSA) as a potent SMS inhibitor. LC–MS-based kinetic assays demonstrated concentration-dependent inhibition with IC_{50} values of 4.5 μM for SMS1 and 6.0 μM for SMS2 respectively. Molecular docking and molecular dynamics simulation analyses revealed strong binding affinity, hydrogen-bonding interactions, and remarkable conformational stability of 9Z-DSA within the enzyme's active site. These findings highlight 9Z-DSA as a promising natural molecule capable of modulating SMS activity, suggesting its nutraceutical relevance for the prevention and management of obesity-related metabolic dysfunctions and hepatic steatosis.

Chapter 4 introduces the green synthesis and functional characterization of hijiki-derived carbon dots (H-CDs), representing a novel class of bioactive nanomaterials. The H-CDs were synthesized via a one-step hydrothermal process using *H. fusiformis* biomass and exhibited strong blue luminescence (λ_{em} = 407 nm, quantum yield = 8.7%), ultrasmall particle size (0.85–3 nm), and abundant surface functionalities such as hydroxyl, carboxyl, and amino groups. The optical and structural features of H-CDs conferred excellent water solubility, colloidal stability, and biocompatibility. *In vitro* studies demonstrated significant inhibition of SMS1 and SMS2 activity, pointing toward enzyme-targeted biofunctionality of these nanomaterials.

Chapter 5 presents a systematic evaluation of various free fatty acids for their inhibitory effects on SMS activity. Through a combination of *in vitro* enzymatic assays, molecular docking, and molecular dynamics simulations, pentadecanoic acid (C15:0) was identified as a potent inhibitor of both SMS isoforms. Docking studies revealed strong non-covalent interactions between C15:0 and key catalytic residues in SMS1 and SMS2, while dynamic simulations confirmed stable enzyme-ligand interactions over time. These results underscore the potential of medium-chain saturated fatty acids as natural regulators of sphingomyelin metabolism.

Chapter 6 shows that dietary supplementation of C15:0 in obesogenic mice confers broad protection to the heart, liver, and spleen by counteracting high-fat–induced weight gain, lipid dysregulation, and proinflammatory gene expression. C15:0 restores membrane lipid composition, reduces steatosis and pathological tissue remodeling, and fosters reparative immune profiles, supporting odd-chain saturated fatty acids particularly C15:0 as bioactive nutrients that can alleviate cardiometabolic and inflammatory disorders and justify revisiting current saturated fat guidelines.

Overall, this thesis establishes an integrated analytical, biochemical, and computational framework for characterizing sphingomyelin synthase activity and uncovering natural inhibitors. Through the discovery of bioactive molecules, including odd-chain saturated fatty acids such as pentadecanoic acid, and multifunctional carbon-based nanomaterials derived from *Hizikia fusiformis*, the work reveals marine and diet-derived modulators of sphingolipid metabolism with both cellular and systemic efficacy. Collectively, these findings advance marine-derived therapeutics and nutraceutical strategies, offering sustainable and physiologically relevant approaches to modulate sphingolipid-driven metabolic disorders.