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Title	Discovery and Characterization of a Novel Bioactive Lectin from Marine Sponge Aulospongus sp.
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Degree Grantor	北海道大学
Degree Name	博士(水産科学)
Dissertation Number	甲第16715号
Issue Date	2026-03-25
DOI	https://doi.org/10.14943/doctoral.k16715
Doc URL	https://hdl.handle.net/2115/99560
Type	doctoral thesis
File Information	Nasir_Sobia_summary.pdf, 全文の要約

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Dissertation Summary

博士の専攻分野の名称：博士（水産科学）　　氏名：NASIR Sobia (ナシル ソビア)

学 位 論 文 題 目

Discovery and Characterization of a Novel Bioactive Lectin from Marine Sponge *Aulospongus* sp.

（*Aulospongus* 属海綿に由来する新規生物活性レクチンの発見とその構造・機能の包括的解析）

A novel, cell-invading, cytotoxic, and N-acetyl sugar-specific Palauan marine sponge lectin, RaspL (Raspailiidae *Aulospongus* sp. lectin), was identified through sequential screening of aqueous crude extracts obtained from deep-sea sponges collected in Japan and Palau. The screening pipeline included rabbit erythrocyte hemagglutination, HeLa cell cytotoxicity, and cell invasion assays.

RaspL exhibits variable molecular masses depending on both the treatment conditions and analytical techniques, including sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), size-exclusion chromatography (SEC), and mass spectrometry (MS). SDS-PAGE indicates a >100 kDa oligomeric form of RaspL under native conditions, which dissociates into ~14 kDa protomers upon denaturation. SEC reveals a single peak at ~94 kDa, whereas deconvoluted mass spectra display six major peaks ranging from 13.9 to 14.6 kDa. Collectively, these findings support the presence of heterooligomeric isoforms of RaspL.

RaspL displays remarkable biochemical and physicochemical properties. A concentration as low as 146 ng/mL was sufficient to agglutinate rabbit erythrocytes. It preferentially binds N-acetylated sugars, including N-acetyl-D-galactosamine (GalNAc), N-acetylneuraminic acid

(NANA), and N-acetyl-D-glucosamine (GlcNAc), with the highest affinity toward GalNAc, as reflected by minimum inhibitory concentrations (MICs): GalNAc (4.2 mM) > NANA (16.7 mM) > GlcNAc (33.3 mM). The lectin remains stable across a broad range of temperatures (40–100 °C for 90 min) and pH values (3.0–12.0).

Structurally, RaspL displays exceptional features. *De novo* peptide mapping using liquid chromatography-tandem mass spectrometry (LC-MS/MS) revealed two major 127- and 133-residue contigs with heterogeneities. Notably, RaspL shows no similarity to any known proteins in the NCBI protein blast database, suggesting a previously uncharacterized lectin. Cryo-electron microscopy (Cryo-EM) uncovered its unusual tetrahedral dodecameric assembly composed of tightly packed β-sheets with deeply buried termini, accounting for its high thermal stability. The presence of twelve GalNAc-binding pockets (one per monomer) may contribute to its strong sugar-binding avidity. No substantial conformational changes were observed upon GalNAc binding, highlighting the robustness of its binding domains.

Functionally, RaspL demonstrates potent cytotoxicity against HeLa cells, with an IC_{50} of 4.36 μg/mL, markedly stronger than that of concanavalin A (IC_{50} of 19.08 μg/mL). Confocal microscopy further confirms its cellular internalization and accumulation in the Golgi apparatus, suggesting that its cytotoxic mechanism likely originates from this organelle. To further explore the molecular basis of RaspL-induced cytotoxicity, transcriptomic (RNA-seq) analysis was performed. Differential gene expression profiling revealed significant modulation of genes associated with Golgi organization, glycosylation, vesicle-mediated transport, and endomembrane trafficking. In addition, transcriptional changes related to the endoplasmic reticulum organization, mitochondrial function, and cellular stress responses were also observed, indicating coordinated involvement of multiple organelles.

Collectively, these transcriptomic patterns are consistent with a Golgi-centered stress response accompanied by broader endomembrane and mitochondrial adaptation rather than

acute organelle collapse. While multiple cell death-related pathways were transcriptionally represented, the RNA-seq data did not support exclusive activation of a single, well-defined death mechanism under the examined conditions, instead suggesting engagement of multimodal stress-responsive and pro-death signaling pathways depending on stress intensity and duration.

Targeted RT-qPCR analysis of selected apoptosis-, autophagy-, and stress-related genes further supported the context-dependent nature of RaspL-induced cytotoxicity. Gene expression changes varied with dose and exposure time and were not characterized by sustained transcriptional activation of a single canonical cell death program. Instead, the RT-qPCR data suggest a dynamic transcriptional response that is consistent with stress-associated modulation rather than uniform engagement of a defined apoptotic or autophagic pathway.

Ongoing work in our group aims to elucidate the detailed mechanism of cytotoxicity, complete monomer-level sequence decoding, and characterize oligomeric ligand-bound complexes. Additionally, the evaluation of other cell-surface receptors coupled with the comprehensive characterization of endocytic routing, time-based and dose-dependent molecular dynamics, organelle stress cross-talks, and multiple cell death modalities will further strengthen the understanding and scope of RaspL in oncological applications.

Overall, the unique sequence and structure of RaspL, its strong N-acetyl sugar-binding preference, potent cytotoxicity, intracellular trafficking to the Golgi, and exceptional stability highlight its potential as a valuable tool in biomedical research, particularly in glycobiology and cancer cell biology.