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学位論文内容の要旨
Dissertation Abstract

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）

Marine sponges are a rich source of biologically active lectins with distinctive structural and functional properties. This study reports the discovery and comprehensive characterization, including physicochemical, biochemical, structural, and functional features, of a novel cell-invading, cytotoxic, and N-acetyl sugar-specific lectin, RaspL, isolated from a Palauan marine sponge belonging to the family Raspailiidae, using a combination of chromatographic techniques.

Biochemical analyses indicated that RaspL exists as a heterooligomeric protein complex with variable apparent molecular masses depending on analytical conditions. SDS-PAGE revealed an oligomeric species exceeding 100 kDa under native conditions that dissociated into ~14 kDa protomers under denaturing conditions. Size-exclusion chromatography showed a single peak of approximately 94 kDa, while mass spectrometry identified six closely related monomeric isoforms (13.9–14.6 kDa), collectively supporting the presence of heterooligomeric assemblies. RaspL exhibited strong hemagglutination activity at a final concentration of 146 ng/mL and showed pronounced specificity toward N-acetylated sugars. Inhibition assays demonstrated the highest affinity for N-acetyl-D-galactosamine (GalNAc), followed by N-acetylneuraminic acid (NANA) and N-acetyl-D-glucosamine (GlcNAc). The lectin displayed exceptional physicochemical stability, retaining activity over a wide range of temperatures (40–100 ℃) and pH values (3.0–12.0).

Structural analyses revealed several distinctive features of RaspL. LC-MS/MS-based *de novo* peptide mapping yielded two major 127- and 133-residue contigs exhibiting sequence heterogeneity, with no detectable similarity to known proteins, indicating that RaspL represents a previously uncharacterized lectin. Cryo-electron microscopy revealed a unique tetrahedral dodecameric architecture composed of tightly packed, β-sheet-rich monomers, with deeply buried termini that likely contribute to its notable thermal stability. The presence of twelve GalNAc-binding pockets (one per monomer) may contribute to the lectin's high sugar-binding avidity. Notably, GalNAc binding did not induce major conformational changes, suggesting a pre-set binding interface provided in the apo form that allows quick, reversible recognition.

Functionally, RaspL exhibited potent cytotoxicity against HeLa cells (IC_{50} of 4.36 μg/mL), exceeding that of the representative plant lectin concanavalin A (IC_{50} of 19.08 μg/mL). Confocal microscopy confirmed cellular internalization and accumulation within the Golgi apparatus, suggesting a Golgi-centered mode of action. Transcriptomic profiling by RNA sequencing revealed significant modulation of genes involved in Golgi organization, glycosylation, vesicle trafficking, and endomembrane transport, along with changes related to endoplasmic reticulum function, mitochondrial activity, and cellular stress responses. These patterns suggest a coordinated, multi-organelle stress response rather than acute organelle collapse. Although transcripts associated with apoptosis, autophagy, and other cell death pathways were detected, neither RNA-seq nor targeted RT-qPCR analyses supported sustained activation of a single canonical death mechanism. Instead, RaspL-induced cytotoxicity appears to involve dynamic, dose- and time-dependent engagement of multimodal stress-responsive and pro-death signaling pathways.

In conclusion, RaspL is a structurally unique and highly stable marine sponge lectin with strong N-acetyl sugar specificity, potent cytotoxic activity, and distinctive Golgi-targeted intracellular trafficking. These properties underscore its potential as a valuable molecular tool in glycobiology and cancer cell research and provide a foundation for future mechanistic and therapeutic studies.