学 位 論 文 の 要 旨

論文題目　Isolation, Characterization, and Applications of Lectins from Edible Red Algae
（食用紅藻由来レクチンの単離、性状評価および応用に関する研究）

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The ocean is a vast and diverse ecosystem that supports a plethora of marine species. The increasing global demand for food and the declining availability of traditional sources of protein have highlighted the need to explore alternative sources of sustainable food. Marine algae comprise an important bioactive compound component that offers nutritional and health benefits. While many of these species are yet to be explored, only a limited number have been utilized as viable food sources for human consumption. Studies indicate that the consumption of seaweed confers significant bioactivity effects such as anti-inflammatory, antioxidant, anticancer, and antiviral properties. Besides, marine algae are widely acknowledged as significant repositories of potential novel therapeutic compounds. Amongst compounds with notable biochemical activity, lectins have emerged as an essential class and turned into a hot issue due to their unique and algae-inherent properties. Lectins are proteins that exhibit a particular affinity for certain sugar compounds that are found in abundance throughout nature. They serve critical functions as recognition molecules in interactions among cells or between cells and the extracellular matrix making them valuable tools for their detection and characterization in various biological systems. Although lectins have been shown to exhibit diverse functional activities, conclusive research regarding their effect on human physiology, particularly those of plant origin, remains a subject of intensive investigation. Lectins are a vital component of biologically active foods in the diet. The ubiquitous presence of lectins means that most plant-based food contains substantial amounts of lectins with remarkable biological activities on gut function. Hence, their physiological functions after consumption are receiving so much attention, particularly the potential for application development they may provide. Thus, this research generally aims to (1) isolate and characterize lectins from edible red alga *Callophyllis japonica*; (2) examine the resistance of algal lectin to several digestive enzymes and its behavior after oral ingestion; and (3) assess the impact of various recombinant enzymes, including agarase, κ-carrageenase, and ι-carrageenase, on the efficiency of lectin extraction from red algae for further application.

(1) Isolation and Characterization of Lectins from Edible Red Alga *Callophyllis japonica*

Callophyllis japonica, a red algae species, is highly valued for its culinary uses and has been found to have various bioactive compounds such as polysaccharides, phenolic compounds, carotenoids, and fatty acids. The screening of lectins in marine algae recently revealed that the extract obtained from *C. japonica* exhibited potent hemagglutination activity. The isolation of lectins from the edible red seaweed *C. japonica* performed various purification processes such as extraction with PBS, ammonium sulfate precipitation, anion exchange

chromatography, gel filtration, and reverse-phase HPLC. The purified lectin (CJL) showed high hemagglutination activity and was identified as a single protein band at approximately 8 kDa and 10 kDa in non-reducing and reducing SDS-PAGE, respectively. The purification process yielded a total of 0.052 mg from 50 g of dried algal material. The CJL demonstrated high thermal and pH stability, with no notable differences observed in hemagglutination activity when exposed to temperatures up to 80°C and pH levels ranging from 4 to 10. Moreover, CJL does not require divalent cations for hemagglutination activity. To further characterize the CJL, its binding specificity to different carbohydrates was investigated. Monosaccharides and disaccharides tested did not have any inhibitory effect against the hemagglutination activity of CJL, while inhibition of glycoproteins such as bovine submaxillary mucin (BSM), asialo-BSM, porcine thyroglobulin (PTG), asialo-PTG, asialo-fetuin, and asialo-transferrin was observed. It suggested that CJL demonstrated a preference for glycans bearing galactose residue at non-reducing terminus because it tended to be inhibited by asialo-derivatives.

(2) The resistance of algal lectin to digestive enzymes and its behavior after oral ingestion

The recombinant form of known red algal lectins including *Meristotheca papulosa* lectin (MPL-1), *Eucheuma serra* lectin (ESA-2), and *Hypnea japonica* lectin (HypninA-2) were prepared using an *E. coli* expression system. The digestion test was performed by incubating the lectins with artificial gastric juice containing pepsin (pH 2.0) and intestinal fluid containing trypsin and chymotrypsin (pH 7.0) for varying durations. These were tested also with several protease enzymes such as actinase, proteinase K, and papain. The stability of the lectins was evaluated using hemagglutination activity and the signal intensity of the correspondent band in SDS-PAGE as indicators, compared with the control plant lectins including soybean agglutinin (SBA), *Agaricus bisporus* agglutinin (ABA), and kidney bean *Phaseolus vulgaris* lectin (PHA-E_4), which are known as a digestion-resistant lectin. Results showed that the recombinant MPL-1 (rMPL-1) from the algal species *Meristotheca papulosa* under the jacalin-related lectin (JRL) exhibited a notable resistance to proteolytic digestion, as demonstrated by their considerable stability during peptic and tryptic digestion. In addition, even when exposed to enzymes such as actinase E, proteinase K, and papain, rMPL-1 remained unchanged and retained its hemagglutination activity after 24 hours of incubation. Interestingly, other lectins from the JRL family, namely Griffithsin (rGRFT), and lectin from Banana (BanLec) were attained to retain their activity after digestion. It suggested that the lectins belonging to the JRL family have digestion-resistant structures in common and that they could reach the intestine with their activity after ingestion. To evaluate functionality in the intestine, the proliferation-inhibitory effect of *M. papulosa* lectin was examined *in vitro* on HT29 cell, a human colon cancer-derived cell line. Lectin fraction of *M. papulosa* effectively inhibited cell proliferation with IC_{50} of 4.5 μg/mL. Its anti-proliferative effect was canceled with yeast mannan, the inhibitory glycoprotein against *M. papulosa* lectin. This finding suggests that *M. papulosa* lectin inhibited the proliferation of HT-29 by binding to glycans on the cell surface. Thus, the robustness and anti-cancer ability of *M. papulosa* lectin may provide valuable insights for the development of functional foods and therapeutic agents for cancer treatment.

(3) Impact of various algal polysaccharide-digestive enzymes, including agarase, κ-carrageenase, and ι-carrageenase

The extraction yields of seaweed bioactive compounds have typically been low and subject to variability across studies, suggesting that the optimization of extraction protocols presents an opportunity for improvement. Hence, the current investigation aims to assess the impact of various algal polysaccharide-digestive enzymes, including agarase, κ-carrageenase, and ι-carrageenase, on the efficiency of protein extraction from red algae. Furthermore, the outcomes of this study may establish a basis for the utilization of sustainable seaweed protein extraction methods, ultimately contributing to food security, while also considering the economic feasibility and scalability of the approach. The study was performed by constructing recombinant enzymes of agarase (rAgaAc) derived from *Zobellia galactanivorans*, κ-carrageenase (rCgkA) from *Pseudoalteromonas carrageenovora*, and ι-carrageenase (rCgiA) from *Alteromonas macleodii* using an *E. coli* expression system. The enzymatic activity was measured using the 3,5-dinitrosalicylic acid method and the biochemical characterization of recombinant enzymes was investigated. These recombinant enzymes were then used to assess protein extraction efficiency by evaluating the protein yield and viscosity. Recombinant enzymes AgaAc, CgkA, and CgiA were purified and expressed in *E. coli* with an apparent molecular weight of around 33 kDa, 40 kDa, and 55 kDa, respectively. The rAgaAc enzyme is a β-agarase with an optimum temperature and pH of 30°C and 9.0, respectively. Moreover, rCgiA and rCgkA displayed maximal activity at 40°C, and both showed stability across a wide range of relatively neutral pH from 6.0-10.0. These recombinant carrageenases, on the other hand, are more alkaline than most reported carrageenases. Notably, the addition of recombinant enzymes to the extract significantly decreased the viscosity. On the other hand, a remarkable increase in protein yield was observed in *Gracilaria textorrii, Chondracanthus tenellus,* and *Callophyllis japonica* treated with rAgaAc, rCgkA, and the mix of the three enzymes, respectively, and the same trend in all enzymes pre-treated extracts in hemagglutination activity. This suggests that the enzymatic pre-treatment of algae is significantly effective in increasing the utilization potential of algal proteins, especially lectins. Hence, the characteristics of these enzymes are valuable in industrial applications due to their energy-saving actions, subsiding cost, and application in biomass saccharification.

The nutritional richness of seaweed products due to their functional qualities and biochemical composition renders them economically viable. Moreover, the seaweed-derived proteins growing market demand and projected to become a limiting nutrient in the future presents a compelling exploration of alternative sources and large-scale cultivation techniques to address the increasing consumer demand and minimize potential environmental impacts on marine ecosystems. The isolation and characterization of lectins have important implications in several fields, including biotechnology, food science, and pharmacology. By understanding the properties of lectins and their interactions with carbohydrates, researchers can develop new therapeutic agents, identify potential allergens and toxins in foods, and explore new food sources that are rich in lectins and other beneficial compounds.