論　文　内　容　要　旨

Capture and neutralization of SARS-CoV-2 and influenza virus by algae-derived lectins with high-mannose and core fucose specificities

（高マンノースおよびコアフコース特異性を有する藻類由来レクチンによる SARS-CoV-2およびインフルエンザウイルスの捕捉と中和反応）

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is an enveloped virus with a diameter of about 100nm, bearing surface (S) proteins on its surface. The S proteins form homotrimers that protrude from the envelope as spikes. S proteins are predicted to have 22 N-glycosylation sites and at least two O-glycosylation sites, and the attached N-glycans have been confirmed to be both the complex and high-mannose (HM) types. The glycan chains of S proteins are not only crucial for protein conformation formation, but from the virus's point of view, they also serve as a shield that protects the virus from antibodies and other substances and are essential molecules for the survival and function of the virus. In this respect, glycan-binding proteins and lectins that target glycans could serve as viral growth inhibitors and diagnostic tools.

We have isolated and analyzed lectins from marine algae and have shown that lectins from algae have unique molecular structures and carbohydrate-binding specificities that differ from those of known lectins from other groups of organisms. Currently, we have an algal lectin library containing more than 50 species of lectins and their recombinants. Among them, BCA, ESA-2, KAA-1, and KAA-2 were reported to be potent inhibitors of influenza virus growth. In addition, BCA and OAA also inhibited HIV-1 entry into host cells. In this study, we report algal-derived lectins' neutralizing and trapping activity against SARS-CoV-2.

The SARS-CoV-2/JP/Hiroshima-46059T/2020 strain was grown in VeroE6/TMPRSS2 cells (African green monkey kidney-derived cultured cells expressing human TMPRSS2) and used as the experimental virus. All experiments using infectious SARS-CoV-2 were performed under Biosafety Level 3 (BSL3) containment measures at the P3 experiment facility of Hiroshima University.

Seven of the nine algal lectins used in this study, OAA, KAA-1, ESA-2, BCA, BPL17, MPL-1, and CV-N, bind specifically to HM-type N-glycans; MVL binds weakly to complex-type N-glycans in addition to HM-type N-glycans; Hypnin-A2 binds to the complex-type N-glycan core fucose. All lectins were expressed and purified in *E. coli*.

Three lectins (OAA, KAA-1, and HypninA-2) were immobilized on Ni-NTA monolithic silica spin columns to create lectin columns and SARS-CoV-2 column binding experiments were performed. The column was loaded with SARS-CoV-2 and centrifuged through the column. The column was then washed with saline and eluted with 0.4% SDS. Finally, all residual viral genome on the column was recovered with the protein denaturing agent Trizol. Because of the significant inter-individual differences in the lectin columns, the results for each column are denoted individually as Experiment 1 and Experiment 2; for Experiment 1 on the OAA column, the flow-through fraction was 29.0%, the wash fraction was 0.1%, the eluted fraction was 38.9%, and the residual fraction was 3.0%. These indicate that a high percentage of the virus is present in the elution fraction, excluding the flow-through fraction that did not bind to the column. In Experiment 2, the elution fraction was as high as 98%. This suggests that SARS-CoV-2 binds to OAA. Similar results were obtained with other KKA-1 and HypninA-2 columns. In the lectin-unattached columns, most of the virus was present in the flow-through fraction, indicating minimal binding to the

column itself.

Next, neutralization tests were performed using the above three lectins plus six more, for a total of nine lectins; KAA-1 and ESA-2 showed neutralizing activity, with infectivity reduced to less than 1/10. However, OAA, which was found to bind to SARS-CoV-2 from column experiments, did not neutralize SARS-CoV-2. HypninA-2 also has SARS-CoV-2 neutralizing activity.

In addition, a series of lectins were used at the same concentrations to study the neutralization of influenza viruses according to the same method: in the case of KAA-1 and ESA-2, the infectivity of influenza viruses was almost completely neutralized, whereas no neutralization was observed with OAA. On the other hand, HypninA-2, which showed neutralization against SARS-CoV-2, showed almost no neutralization against influenza viruses.

In order to determine whether the neutralizing activity of KAA-1, ESA-2, and HypninA-2 against SARS-CoV-2 is mediated by specific glycans, competition experiments using foreign glycoproteins were conducted. Results showed that these lectins neutralize the virus via HM-type glycans (KAA-1 and ESA-2) or core fucose-type glycans (HypninA-2).

Enriched SARS-CoV-2 was treated with three lectins, OAA, KAA-1, and HypninA-2, and observed under an electron microscope. The virus particles were dispersed in the micrographs of lectin-untreated SARS-CoV-2, but in the lectin-treated samples, the virus particles were aggregated. Therefore, the area of each particle was measured in the micrographs to estimate the number of viral particles contained, and the agglutination occurred with an intensity in the order of HypninA-2 > KAA-1 > OAA.

This study showed that KAA-1 and ESA-2, HM-type glycan-specific lectins, and HypninA-2, a core fucose-specific lectin, have neutralizing activity against SARS-CoV-2. On the other hand, OAA, which was found to bind SARS-CoV-2, showed no neutralizing activity.

Possible neutralization mechanisms are the steric hindrance of the S protein and viral aggregation. In steric hindrance, KAA-1 and ESA-2 bind to high mannose-type sugar chains on the S protein, covering the receptor binding domain (RBD) and reducing infectivity. Therefore, even if OAA binds, steric hindrance does not occur. On the other hand, the extent of viral aggregation may be related to the number of binding sites that lectins have: KAA-1 and ESA-2 have four binding sites per molecule. In contrast, OAA, which showed no neutralizing ability, has only two binding sites per molecule.

Based on the above, the neutralization of SARS-CoV-2 by lectins is thought to occur because factors such as the molecular weight of lectins, the number of binding sites, and the position of the sugar chains on the S protein to which lectins bind are related, causing steric hindrance of the S protein and viral aggregation.

Lectins may provide a new means of detection or protection against not only SARS-CoV-2 but also other coming novel pandemic pathogens. The properties of lectins that bind to sugar chains are expected to facilitate virus enrichment and detection as well as improve protection against viruses. In particular, the application of lectins in virus countermeasures during a pandemic has excellent potential.