Screening potential virus-binding materials from marine alga

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Introduction

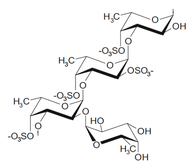

Fig.1 The structure of fucoidan, a representative virus-binding glycan from marine brown seaweeds.

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus invades its host through the interaction of its spike (S) protein with a host cell receptor, angiotensin-converting enzyme 2 (ACE2), and cell surface heparan sulfate (HS) which serves as a co-receptor. Our previous studies demonstrated that heparin exhibited stronger binding capacity to the spike protein than that of less sulfated HS. Moreover, sulfated glycans from marine alga exhibited notable activity in inhibiting the S-protein binding to heparin/heparan sulfate [1-3]. Herein, we screened marine alga-derived glycans for potential virus binding materials and elucidated structure-activity relationship of the glycan-virus binding.

Research Question

We previously reported that two fucoidan extracts from *Saccharina japonica*, RPI 27 and RPI 28, exhibited impressive activities in competing with heparin for S-protein binding [1]. As both fucoidan extracts are mixtures of sulfated glycans with different molecular weights and sulfation levels, it is yet unclear what components in RPI 27/28 are responsible for anti-virus activity. In the current study, with the aim of discovering more efficient virus binding materials from marine alga, we are focusing on the following research questions: 1. What are the sub-fractions in RPI 27 and 28 that contribute to antivirus activity; 2. What is the structure-activity relationship in alga glycan-virus binding; and 3. What other species of marine alga (other than *S. japonica*) can be used to produce virus-binding glycans.

Methods

Our previous experiments showed that both strong anion exchange (SAX) and weak anion exchange (WAX) worked for algal sulfated glycans separation. Nonetheless, SAX is superior in differentiating highly negatively charged components. Therefore, we used a sequential fractionation protocol starting with separating fucoidans using molecular-weight-cut-off (MWCO) centrifugal filters and followed by SAX separation using quaternary ammonium (R-CH_2-N^+ -$(CH_3)_3Cl$) resin. The major subfractions were used in surface plasmon resonance (SPR) binding assays.

Crude fucoidan from *S. Latissima* (a local brown seaweed); RPI 27 and RPI 28 from *S. japonica*

→ Molecular-weight-cut-off (MWCO) fractionation 100KD~30KD~10KD~3KD~

→ SAX sub-fractionation, eluted with NaCl 0.2M(loading) ~0.5M~ 1.0M ~ 2.0M ~ 5.0M

→ The major subfractions were subjected to the SPR competition assay : inhibiting S-protein RBD binding to the heparin chip

Results

Fractionation of Fucoidans

Fig.2 The yields (%) of the fucoidans separated using MWCO-SAX sequential fractionation. The pie charts show the yields of different fucoidans using MWCO method and the column charts show the yields using SAX for sub-fractionation. The marked fractions were further investigated using SPR.

Surface Plasmon Resonance Assay

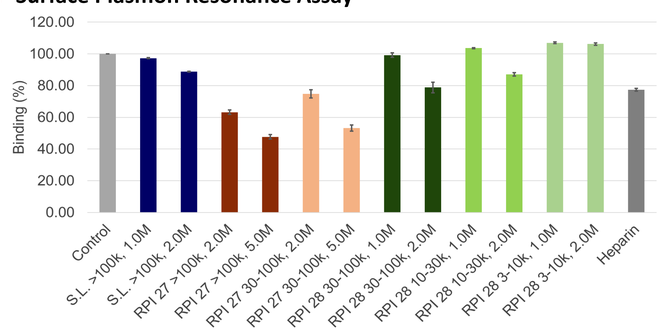

Fig. 3 S-protein RBD binding to chip-surface heparin inhibited by different fucoidan fractions or heparin at conc. of 62.5 ng/mL in solution. The sub-fractions of RPI 27 showed stronger inhibition compared to heparin in solution. The fucoidans interacted with RBD and inhibited RBD binding to the chip-surface heparin. Therefore, the inhibitory effects of different fucoidans reflect their virus-binding abilities.

Conclusion

1. Fractionation of fucoidan from different algae species yielded highly different fractions using the MWCO-SAX sequential procedure. RPI 27 from *Saccharina Japonica* contains highly negatively charged fucoidans that require a high NaCl concentration to recover from SAX.

2. From the current data, fractions with both higher Mw and sulfation level (indirectly reflected by the salinity of NaCl used in WAX elution) would result in higher activity in binding to S-protein RBD.

3. Fractions with similar Mw range and sulfation level from the same algae species exhibited comparable virus-binding activity, e.g., RPI 27 $_{30\text{-}100k,\ 2.0M}$ vs. RPI 28 $_{30\text{-}100k,\ 2.0M}$. However, the fractions from different species are less comparable, e.g., S.L. $_{>100k,\ 2.0M}$ vs RPI 27 $_{>100k,\ 2.0M}$.

4. The low Mw fraction RPI 28 $_{3\text{-}10k,\ 2.0M}$ did not show promising competitive binding to virus even though it was eluted at a high salinity.

5. Other algal glycans, such as rhamnan sulfate from green alga and carrageenan from red alga, may also be included in future screening. More detailed structural elucidation of the glycans, such as monosaccharide composition, glycosidic linkage, sulfation position, degree of branching, etc., are needed to create a more comprehensive structure-activity relationship.

Acknowledgement

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References

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