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# The sweet quartet: Binding of fucose to the norovirus capsid

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ABSTRACT

Human noroviruses bind histo-blood group antigens (HBGAs) and this interaction is thought to be important for an infection. We identified two additional fucose-binding pockets (termed fucose-3/4 sites) on a genogroup II human (GII.10) norovirus-protruding (P) dimer using X-ray crystallography. Fucose-3/4 sites were located between two previously determined HBGA binding pockets (termed fucose-1/2 sites). We found that four fucose molecules were capable of binding altogether at fucose-1/2/3/4 sites on the P dimer, though the fucose molecules bound in a dose-dependent and step-wise manner. We also showed that HBGA B-trisaccharide molecules bound in a similar way at the fucose-1/2 sites. Interestingly, we discovered that the monomers of the P dimer were asymmetrical in an unliganded state and when a single B-trisaccharide molecule bound, but were symmetrical when two B-trisaccharide molecules bound. We postulate that the symmetrical dimers might favor HBGA binding interactions at fucose-1/2 sites.



## Introduction

Human noroviruses are the dominant cause of acute gastroenteritis. Noroviruses are genetically and antigenically distinct with two main genogroups (GI and GII) causing the majority of infections in humans. The norovirus capsid protein is divided into the shell (S) and protruding (P) domains, the latter of which is further subdivided into P1 and P2 subdomains (Prasad et al., 1999). Noroviruses bind histo-blood group antigens (HBGAs), which are polymorphic carbohydrate structures present as free antigens in saliva and found on the surface of various epithelia. Interestingly, HBGA binding was found to enhance norovirus infection in cell culture (Jones et al., 2014). Noroviruses bind HBGAs on the P domain, where GI noroviruses have a monomeric interaction with HBGAs and GII noroviruses have a dimeric interaction (Hansman et al., 2011; Cao et al., 2007; Bu et al., 2008). At least nine different HBGA types have been recognized to bind to noroviruses and these are typically grouped into either ABH or Lewis types that are distinguished by the different connections of a fucose moiety. The ABH types are characterized by an α-L-fucose-(1-2)-β-D-galactose connection, whereas the Lewis types contain an α-L-fucose-(1-3)/(1-4)-β-D-*N*-acetyl-glucosamine. The GII noroviruses primarily interact with the ABH-fucose, Lewis-fucose, or a combination of both. The fucose moiety of the HBGAs is usually held firmly by a common set of GII P domain residues, whereas the other saccharide units are held less firmly with variable residues and water-mediated interactions (Hansman et al., 2011; Singh et al., 2015).

In this study, we investigated a concentration-dependent binding of L-fucose-monosaccharide (fucose) and B-trisaccharide (B-tri) to the GII genotype 10 (GII.10) norovirus P dimer using X-ray crystallography. We identified two additional fucose-binding pockets and showed that four fucose moieties were bound per dimer. We also found that fucose and B-tri bound in a dose-dependent and step-wise manner.

## Results and discussion

Molecular replacement indicated a P dimer in space group $P2_1$ for all datasets. Data statistics are shown in Tables 1 and 2. All P domain complexes were reminiscent of the previously published unliganded GII.10 P domain structure (Fig. 1A) (Hansman et al., 2011). Co-crystallization at 300 mM and 150 mM of fucose revealed four clearly discernable patches of fucose electron density

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**Table 1**
Data collection and refinement statistics of GII.10 P domain fucose complex structures.

| | GII.10-Fucose$^{300\ mM}$ (4Z4R) | GII.10-Fucose$^{150\ mM}$ (4Z4S) | GII.10-Fucose$^{75\ mM}$ (4Z4T) | GII.10-Fucose$^{37.5\ mM}$ (4Z4U) | GII.10-Fucose$^{19\ mM}$ (4Z4V) | GII.10-Fucose$^{4.7\ mM}$ (4Z4W) |
|---|---|---|---|---|---|---|
| Data collection | | | | | | |
| Space group | $P2_1$ | $P2_1$ | $P2_1$ | $P2_1$ | $P2_1$ | $P2_1$ |
| Cell dimensions | | | | | | |
| *a*, *b*, *c* (Å) | 66.10 78.94 68.89 | 66.09 78.68 68.81 | 65.75 78.85 68.64 | 65.32 78.77 68.51 | 65.11 78.82 68.66 | 65.29 79.34 69.58 |
| α, β, γ (°) | 90 99.84 90 | 90 99.61 90 | 90 99.43 90 | 90 99.52 90 | 90 99.77 90 | 90 100.78 90 |
| Resolution range (Å) | 43.44–1.80 | 43.08–1.80 | 43.42–1.80 | 43.19–1.88 | 43.05–1.80 | 43.41–1.80 |
| | (1.87–1.80)[a] | (1.86–1.80)[a] | (1.86–1.80)[a] | (1.95–1.88)[a] | (1.86–1.80)[a] | (1.86–1.80)[a] |
| $R_{sym}$ | 3.96 (17.11)[a] | 6.39 (29.73)[a] | 4.43 (18.71)[a] | 7.84 (60.84)[a] | 4.9 (38.16)[a] | 7.01 (23.37)[a] |
| $I/\sigma I$ | 21.91 (6.59)[a] | 13.76 (4.06)[a] | 18.25 (5.97)[a] | 11.56 (1.81)[a] | 16.73 (3.02)[a] | 16.62 (5.99)[a] |
| Completeness (%) | 99.26 (95.78)[a] | 99.01 (97.40)[a] | 98.79 (96.05)[a] | 98.92 (95.03)[a] | 98.81 (95.10)[a] | 98.66 (94.29)[a] |
| Redundancy | 3.4 (3.3)[a] | 3.2 (3.2)[a] | 3.1 (2.8)[a] | 3.0 (2.9)[a] | 2.9 (2.9)[a] | 3.0 (2.8)[a] |
| Refinement | | | | | | |
| Resolution range (Å) | 43.44–1.80 | 43.08–1.80 | 43.42–1.80 | 43.19–1.88 | 43.05–1.80 | 43.41–1.80 |
| No. of reflections | 64050 | 63885 | 63487 | 54695 | 62731 | 63970 |
| $R_{work}/R_{free}$ | 0.14/0.17 | 0.14/0.18 | 0.15/0.17 | 0.16/0.20 | 0.15/0.18 | 0.15/0.19 |
| No. of atoms | 5690 | 5664 | 5535 | 5366 | 5454 | 5643 |
| Protein | 4893 | 4847 | 4767 | 4768 | 4790 | 4806 |
| Ligand/ion | 64 | 80 | 72 | 58 | 39 | 23 |
| Water | 733 | 737 | 696 | 540 | 625 | 814 |
| Average *B* factors (Å$^2$) | | | | | | |
| Protein | 18.70 | 17.00 | 20.00 | 27.40 | 24.10 | 16.10 |
| Ligand/ion | 18.70 | 17.20 | 24.50 | 31.20 | 26.50 | 20.40 |
| Water | 30.70 | 29.40 | 31.10 | 35.80 | 34.60 | 28.40 |
| RMSD | | | | | | |
| Bond length (Å) | 0.014 | 0.015 | 0.008 | 0.008 | 0.011 | 0.008 |
| Bond angle (°) | 1.42 | 1.43 | 1.14 | 1.06 | 1.29 | 1.15 |

Each data set was collected from single crystals, respectively.
[a] Values in parentheses are for highest-resolution shell.

**Table 2**
Data collection and refinement statistics of GII.10 P domain B-tri complex structures.

| | GII.10-Btri$^{30\ mM}$ (4Z4Z) | GII.10-Btri$^{7.5\ mM}$ (4Z4Y) |
|---|---|---|
| Data collection | | |
| Space group | $P2_1$ | $P2_1$ |
| Cell dimensions | | |
| *a*, *b*, *c* (Å) | 66.83 78.29 71.63 | 65.12 78.67 69.77 |
| α, β, γ (°) | 90 103.35 90 | 90 100.36 90 |
| Resolution range (Å) | 42.86–1.80 | 43.2–1.80 |
| | (1.86–1.80)[a] | (1.86–1.80)[a] |
| $R_{sym}$ | 6.86 (50.46)[a] | 4.78 (20.84)[a] |
| $I/\sigma I$ | 11.57 (2.14)[a] | 18.00 (6.12)[a] |
| Completeness (%) | 98.88 (95.13)[a] | 99.08 (95.54)[a] |
| Redundancy | 3.0 (3.0)[a] | 2.3 (3.1)[a] |
| Refinement | | |
| Resolution range (Å) | 42.86–1.80 | 43.20–1.80 |
| No. of reflections | 65787 | 63976 |
| $R_{work}/R_{free}$ | 0.16/0.19 | 0.14/0.18 |
| No. of atoms | 5426 | 5457 |
| Protein | 4805 | 4809 |
| Ligand/ion | 94 | 69 |
| Water | 527 | 579 |
| Average *B* factors (Å$^2$) | | |
| Protein | 21.70 | 18.30 |
| Ligand/ion | 25.30 | 21.80 |
| Water | 28.00 | 27.60 |
| RMSD | | |
| Bond length (Å) | 0.007 | 0.010 |
| Bond angle (°) | 1.06 | 1.24 |

Each data set was collected from single crystals, respectively.
[a] Values in parentheses are for highest-resolution shell.

(Fig. 1A and B). Two electron density patches corresponded to fucose molecules at the HBGA binding sites as previously described (hereafter termed fucose-1 and fucose-2 sites) (Hansman et al., 2011). Two additional electron density patches, representing two fucose molecules (termed fucose-3 and fucose-4 sites), were located between fucose-1/2 sites. At 300 mM and 150 mM of fucose, the four-fucose electron density patches were equivalent, suggesting a similar occupancy at fucose-1/2/3/4 sites (Fig. 1B). At 75 mM of fucose, the electron density patches of fucose at fucose-1/2/3/4 sites were apparent, although fucose-3/4 electron densities were less distinct than the fucose-1/2 sites, indicating a weaker fucose binding at fucose-3/4 sites (Fig. 1B). At 37.5 mM of fucose, only fucose molecules at fucose-1/2 sites bound to the P dimer, since the electron densities at fucose-3/4 sites were lacking and as such fucose was not modeled into the structure (Fig. 1B). At 19 mM and 4.7 mM of fucose, only fucose-1 site was occupied (Fig. 1B).

These data indicated that fucose bound to the P dimer in a dose dependent manner and that binding occurred through a three-step binding process, i.e., firstly fucose-1 site, followed by fucose-2 site, and then simultaneously fucose-3/4 sites. Our data also indicated that fucose-3/4 sites were only occupied after binding at fucose-1/2 sites. Taken together, these new findings implied a cooperative binding of the fucose molecules on the P dimer, which might be associated with different affinities at fucose-1, fucose-2, and fucose 3/4 sites.

At fucose-1/2 sites, the fucose molecules were held at the dimeric interface by the regular set of P domain residues (Hansman et al., 2011). Direct interactions were provided with the side chains of Asn355, Asp385, and Arg356, the main chain of Gly451, and a hydrophobic interaction with Tyr452 (Fig. 1C). The