

Studies on interactions of norovirus capsid protein with fucosylated glycans and galactosylceramide as soluble and membrane bound ligands

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Sahlgreńska akademien vid Göteborgs universitet kommer att offentligens försvaras i lokal Hjärtat, Sahlgreńska Universitetssjukhuset, Göteborg, Måndagen den 9 juni 2014 kl 13:00

av

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Avhandlingen baseras på följande delarbeten:

- I. Koppisetty CA, **Nasir W**, Strino F, Rydell GE, Larson G, Nyholm PG. 2010. *Computational studies on the interaction of ABO-active saccharides with the norovirus VA387 capsid protein can explain experimental binding data*. Journal of Computer-Aided Molecular Design 24(5):423-431.
- II. **Nasir W**, Frank M, Koppisetty CA, Larson G, Nyholm PG. 2012. *Lewis histo-blood group a1,3/a1,4 fucose residues may both mediate binding to GII.4 noroviruses*. Glycobiology 22(9):1163-1172.
- III. **Nasir W**, Bally M, Zhdanov VP, Larson G, Höök F. *Interaction of virus-like particles with vesicles containing glycolipids: Kinetics of detachment*. Manuscript.
- IV. **Nasir W**, Bally M, Kunze A, Zhdanov VP, Parra F, Peters T, Höök F, Larson G. *Binding and inhibition studies on interactions of GII.4 norovirus-like particles with membrane bound fucosylated histo-blood group antigens*. Manuscript.
- V. Bally M, Rydell GE, Zahn R, **Nasir W**, Eggeling C, Breimer ME, Svensson L, Hook F, Larson G. 2012. *Norovirus GII.4 virus-like particles recognize galactosylceramides in domains of planar supported lipid bilayers*. Angew Chem Int Ed Engl 51(48):12020-12024.



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Studies on interactions of norovirus capsid protein with fucosylated glycans and galactosylceramide as soluble and membrane bound ligands

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Noroviruses (NVs) are among the most common viral pathogens which target the gastrointestinal (GI) tract and cause severe diarrhea, vomiting and episodes of abdominal cramps with fever. Millions of people around the world get infected with NVs annually, of which 200,000 cases are estimated to be fatal. Yet for decades, the failure of propagating the human NVs in a cell-culture model has hampered NV infection research and consequently treatment and vaccine development. Thus, the research has mainly been focused on epidemiology and studies on the interaction of model virus-like particles (VLPs) with potential host receptors or attachment factors to gain understanding of the first steps of the infection in order to successfully pave the way for effective clinical therapy or prophylaxis. Motivated by this theme, the emphasis of the present work is mainly on protein-carbohydrate interactions of host glycans with NV VLPs. About 80 % of NV outbreaks reported to date are caused by GII.4 genocluster of NVs. Therefore, due to its dominating clinical importance GII.4 NV-like particles and capsid protein dimers are used for *in vitro* and *in silico* studies included in the thesis work.

NVs have been shown to recognize host histo-blood group antigens (HBGAs) as viral receptors or attachment factors. To investigate the molecular details of interactions of GII.4 NVs with a repertoire of fucosylated HBGAs, molecular dynamics studies were initially carried out based on the crystal structure of B-trisaccharide HBA in complex with VA387 GII.4 norovirus P dimer. The results, which were later confirmed by crystallographic studies, could explain, on an atomic level, the binding characteristics from a mutagenesis study carried out earlier on the same NV strain. Along with the modelling studies theoretical binding energies were also estimated for different HBGAs binding to VA387 P dimers. The atomic details of binding modes revealed how a single fucose binding site could exploit two different binding modes of the same glycan. This was supported by a literature review of the occurrence of similar fucose binding sites and modes observed in nature for fucose binding lectins and antibodies.

One of the objectives of the thesis was to understand the dynamics of virus host interactions at the cell surface membrane, as it holds clues to very early steps of virus infection. Therefore, total internal reflection fluorescent microscopy (TIRFM) was developed to study the binding events of glycosphingolipid (GSL)-containing vesicles to single NV like particles bound to a supported lipid bilayer (SLB). The advantage of this single vesicle binding assay is the ability to analyze the attachment-detachment kinetics both in transient and steady state conditions. Therefore, it enabled us, for the first time, to discriminate between compositionally different GSL-containing vesicles based on their detachment activation energy. This relates directly to the binding strength of the virus-vesicle complex thereby providing new insights into the characteristics of binding virus-like particles to various lipid bound glycans. Moreover, the differences in the distribution of detachment energy of activation for different GSL-containing vesicles were also analyzed.

Microdomains or clustered patches of GSLs with or without cholesterol are dynamic integral parts of most of the plasma membranes. Their role has been implicated in virus infection of HIV and influenza virus but not in NVs. However for the first time, NVs were shown to recognize galactosylceramide (GalCer) microdomains in supported lipid bilayers. The atomic details of the binding mode of these interactions are, however, still to be clarified.

In conclusion, the thesis describes details of viral protein - host carbohydrate interactions at the molecular level, of relevance for understanding virus infection and design of novel anti-viral strategies.

Keywords: norovirus, molecular dynamics, molecular docking, total internal reflection fluorescent microscopy, desorption activation energy, virus-host interactions, histo-blood group antigens, fucose

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