Expression And NMR Characterization Of Labelled P-domains Of Emerging Norovirus

Abstract:

Noroviruses are members of the Caliciviridae family and are non-enveloped, single-stranded, positive-sense RNA viruses. Norovirus is the major cause of acute gastroenteritis, particularly affecting vulnerable populations such as children, the elderly, and immunocompromised individuals. With millions of illnesses and thousands of deaths yearly, norovirus poses a significant global health burden. The economic impact is equally staggering, with billions of dollars in costs annually. Additionally, new virus strains emerge every 2-5 years, making outbreaks difficult to contain. Among the various norovirus strains, the GII.4 variant is the most prevalent globally.

The ability of norovirus to infect host cells depends on the interaction between the Protruding domain (P domain) of its capsid protein VP1 and Histo-Blood Group Antigens (HBGAs) on the surface of host cells. Furthermore, previous cell-based studies found, that Human Milk Oligosaccharides (HMOs) prevent norovirus infection to cells, by acting as a decoy receptor and blocking the interaction between HBGAs and human noroviruses. Therefore, understanding the structure of the P domain is essential for the development of effective antiviral drugs and vaccines.

In this study, we successfully expressed and purified the P domain from the GII.4. P16 norovirus strain in *E. coli*. To enhance NMR signal resolution, isotopically labeled samples (^{15}N, ^{2}H, and ^{13}C) were prepared, and multidimensional NMR experiments, along with chemical shift perturbation (CSP)-based NMR experiments, were performed. These experiments will help identify key residues involved in binding to HBGAs and other antiviral molecules, such as analogs of HMOs. By elucidating these interactions, our studies provide crucial insights into the viral infection mechanism and offer valuable information for the development of new therapeutic strategies.