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Structure of a GI.8 norovirus P domain in complex with Lewis epitope saccharides

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1 Introduction

Noroviruses (NoVs) belong to Norovirus genus in the family Caliciviridae and are the most important viral pathogens of epidemics of acute gastroenteritis in humans, causing significant morbidity and mortality worldwide. Its capsid protein is divided into two major domains, the shell (S) and the protruding (P) domains[1]. The P domain plays an important role in virus-host interaction and immune responses[2].

Human NoVs recognize histo-blood group antigens (HBGAs) as receptors for infection. NoVs interact with HBGAs in strain-specific manners[3]. In this study, we determined the crystal structure of the P dimer of Boxer virus (BV), a GI.8 NoV, in complex with Le^b and Le^y tetrasaccharides, respectively. Our results showed that the extended surface loop P from the opposite protomer contributes largely in forming the Le epitope binding site of BV.

2 Experiment

The P domain of Boxer virus capsid as well as mutants were expressed in E. coli BL21 (DE3). The expression and purification method was similar to VA207 P protein as previously reported[4]. P protein was concentrated and crystallized with hanging drop vapor diffusion method with reservoir solution of containing 0.1M LiCl, 18% (w/v) PEG 3,350 and 10% (v/v) 2-Methyl-2,4-Pentanediol (MPD) at 16℃. For complex crystal growth, the P protein was mixed with equal volume of Le^b or Le^y tetrasaccharide to a final molar ratio of 1:40 and then crystallized with seeding technique.

Native and complex crystals were soaked in cryo-protectant before diffraction test and data collection. X-ray diffraction data were indexed, integrated and scaled by HKL2000. The crystal structure of Norwalk virus P protein was used as model by the program Phaser to solve the phases of the Boxer P protein structure. The structures of P protein complexed with Le^b and Le^y tetrasaccharides were solved with the native P protein structure as search model, followed by structure optimization and validation with Coot, PHENIX and Procheck.

The affinity-column purified P particles of Boxer virus were diluted to 0.2 mg/ml and HBGA binding assays were performed as described [4]. A panel of synthetic oligosaccharides and characterized saliva samples of known HBGA phenotypes were used for binding assays.

3 Results and Discussion

The Boxer virus P protein contains two P protomers in an asymmetric unit. Its structure can be divided into P1 and P2 subdomains: the P1 subdomain is a mixed α/β structure, and the P2 subdomain is constituted mainly by a β-barrel structure formed by two twisted antiparallel β sheets.

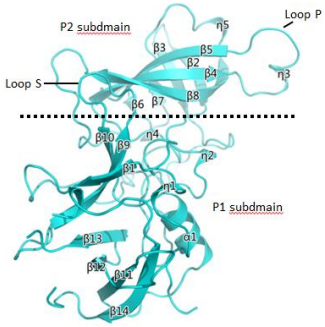

Fig. 1: Structure of Boxer virus P domain monomer

The crystal structures of Boxer P dimers in complex with Le^b and Le^y tetrasaccharide clearly showed that nine participating amino acids from 3 loop regions formed the binding site. Boxer virus interacts with the Le^b and Le^y tetrasaccharides in a way that the β-galactose (Gal) of the precursor vertically occupies the "bottom", which is stabilized by one "wall" (T397) of the binding pocket, while the α-1,3/4 fucose (Fuc), the Le epitope, has contacts with the other "wall" to further stabilize the binding outcomes.

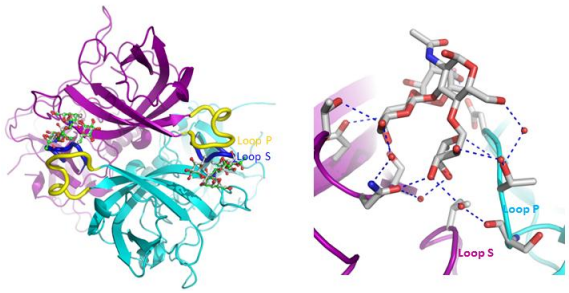

Fig. 2: The structures of the HBGA binding interface

References

[1] Zheng. D *et al.*, *Virology* **346**:312-323 (2006)

[2] Tan, M., and X. Jiang PLoS Pathog **6**:e1000983 (2010)

[3] Huang, P. *et al., J Infect Dis* **188**:19-31 (2003)

[4] Chen, Y. *et al.,* PLoS Pathog **7**:e1002152 (2011)

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