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Strain-specific interaction of a GII.10 Norovirus with HBGAs

Miao Jin [a,1], Ming Tan [b,c,1], Ming Xia [b], Chao Wei [b], Pengwei Huang [b], Leyi Wang [b], Weiming Zhong [b], Zhaojun Duan [a,*], Xi Jiang [b,c,**]

[a] National Institute for Viral Disease Control and Prevention, China CDC, 155 Changbai Road Street, Chang-ping District, Beijing 102206, China
[b] Divisions of Infectious Diseases, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH, USA
[c] Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA

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ABSTRACT

Noroviruses (NoVs), an important cause of gastroenteritis in humans, recognize human histo-blood group antigens (HBGAs) as receptors. The crystal structures of the protruding (P) domain of a GII.10 NoV (Vietnam 026) in complex with various HBGA oligosaccharides were elucidated. However, the HBGA binding profile of this virus remains unknown. In this study, we determined the saliva and oligosaccharide binding profiles of this virus and the roles of amino acids that are involved in HBGA binding. Our data showed that Vietnam 026 bound to all ABO secretor and non-secretor saliva with clear signals detected by monoclonal antibodies against H3, H1, Le^y, Le^a and sialyl Le^a. Mutagenesis study confirmed the binding site determined by the crystallography study, in which single mutations wiped out the binding function. We also identified amino acids surrounding the central binding pocket that may participate in the binding by affecting the HBGA binding specificity of the GII.10 NoV.

Introduction

Noroviruses (NoVs), a group of small-and-round structured RNA viruses constituting *Norovirus* genus in the family *Caliciviridae*, are a major cause of epidemic and sporadic acute gastroenteritis in humans. In the United States, NoVs cause19–21 million cases of acute gastroenteritis annually (Hall et al., 2013; Scallan et al., 2011). Structurally NoVs are non-enveloped viruses with protein capsids that encapsulated a single-stranded, positive-sense, polyadenylated RNA genome of ~7.5 kb. The NoV genome contains three open reading frames (ORFs). ORF1 encodes a large polyprotein that is post-translationally cleaved into six nonstructural proteins, while ORF2 and 3 encodes the major (VP1) and the minor (VP2) structural proteins, respectively.

NoV capsid comprises a single major structural protein (VP1) that is divided into two principal domains, the shell (S) and the protruding (P) domains. While the S domain forms the interior, icosahedral shell, the P domain constitutes the protruding spikes extending from the shell (Prasad et al., 1999). The S and the P domains can be structurally and functionally independent. Expression of the S domain alone results in thin layer, icosahedral S particles, corresponding to interior shell of the capsid without the protruding P domains (Bertolotti-Ciarlet et al., 2002; Tan et al., 2004a). On the other hand, the P domain alone can form different complexes, including 24 mer P particles (Tan et al., 2008a; Tan et al., 2011b; Tan and Jiang, 2005b), 12 mer small P particles (Tan et al., 2011a) and P dimers (Tan et al., 2004a; Tan et al., 2008c). These P domain complexes are interchangeable in certain condition (Bereszczak et al., 2012) and they all bind to histo-blood group antigens (HBGAs) (Tan et al., 2011a; Tan et al., 2004a; Tan and Jiang, 2005b), the viral receptors or attachment factors of NoVs (Tan and Jiang, 2005a, 2007, 2010, 2011). NoV P domain complexes have been useful tools for study of NoV–HBGA interactions (Tan et al., 2004a; Tan et al., 2008b; Tan et al., 2006; Tan et al., 2008c; Tan et al., 2009).

NoVs recognize HBGAs in a strain-specific manner and eight HBGA binding profiles of NoVs have been observed (Huang et al., 2003; Huang et al., 2005). HBGAs are complex carbohydrates present abundantly on mucosal epithelia of gastrointestinal track where they serve as receptors or attachment factors for NoVs to initiate infections. In addition, HBGAs also exist as free antigens in biologic fluids, including blood, saliva and milk. Increasing data showed that NoV–HBGA interactions play an important role in the host susceptibility of NoVs (Frenck et al., 2012; Hutson et al., 2002; Lindesmith et al., 2003; Tan and Jiang, 2010, 2011; Tan et al., 2008b). The HBGA binding sites have been mapped on the tops of the P dimers and the structural bases of NoV–HBGA interactions and have been elucidated in detail by crystallography (Bu et al., 2008; Cao et al., 2007; Chen et al., 2011; Choi et al., 2008; Hansman et al., 2011; Kubota et al., 2012; Shanker et al.,

* Corresponding author. Tel.: +86 10 6358 1342; fax: +86 10 8354 8065.
** Correspondence to: Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229-3039. Tel.: +1 513 636 0119; fax: +1 513 636 7655.
E-mail addresses: zhaojund@126.com (Z. Duan), jason.jiang@cchmc.org (X. Jiang).
[1] These two authors contributed equally to this work.

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2011; Shanker et al., 2014) and by site-directed mutagenesis (de Rougemont et al., 2011; Tan et al., 2008c; Tan et al., 2009). These extensive structural studies provide solid foundation to understand the complex interactions between the diverse NoVs and the polymorphic HBGAs, including ABO, Lewis and secretor antigens (Tan and Jiang, 2010, 2011, 2014).

The association of the host susceptibility of NoVs with their HBGA binding profiles has been shown by the human challenge studies on the prototype Norwalk virus (GI.1) (Hutson et al., 2002; Lindesmith et al., 2003) and the two GII.4 NoVs (Frenck et al., 2012). Similar associations have also been shown through outbreak investigations (Carlsson et al., 2009; Kindberg et al., 2007; Tan et al., 2008b; Thorven et al., 2005). In contrast to the predominant GII.4 NoVs, GII.10 NoVs represent a rare genotype. However, a recent crystallography study showed that a GII.10 NoV, named Vietnam 026, interacts with all five secretor HBGAs, including A, B, H, Le^b, and Le^y antigens (Hansman et al., 2011), raising a question on the spectrum of HBGA-binding profile of this NoV and the association of the spectrum with the host susceptibility and prevalence of this NoV. To address these questions we determined the HBGA-binding specificity of Vietnam 026 through conventional saliva and oligosaccharide-based Elisa and confirmed major data of the crystallography studies with minor discrepancies. In addition, we studied the roles of residues in and around the HBGA binding site by structural based mutagenesis analysis and confirmed the binding site determined by the crystallography study. We also observed that residues around the core binding sites may influence the binding specificity of this NoV.

Results

Production of GII.10 P particles

The P domain of Vietnam 026 (GII.10) was expressed as a GST fusion protein (GST–P) in *E. coli*, which yielded ~10 mg/liter bacteria culture. Digestion of the fusion protein by thrombin resulted in a mixture of P protein (~35 kDa) and GST (~26 kDa) (Fig. 1A, lane C). As expected, gel-filtration analysis of the cleaved proteins revealed two major peaks with molecular weight of ~830 kDa and ~50 kDa, respectively (Fig. 1B), each representing the P particles (~830 kDa) and GST dimers (52 kDa) (Fig. 1A). These data indicated that vast majority of the P protein of Vietnam 026 formed P particles.

Hyperimmune sera against Vietnam 026

Such antisera were produced by immunization of the purified P particles to mice ($n=4$, see Materials and methods). Highly reactive, GII.10 NoV antisera with an average titer of ~1:500,000 were obtained

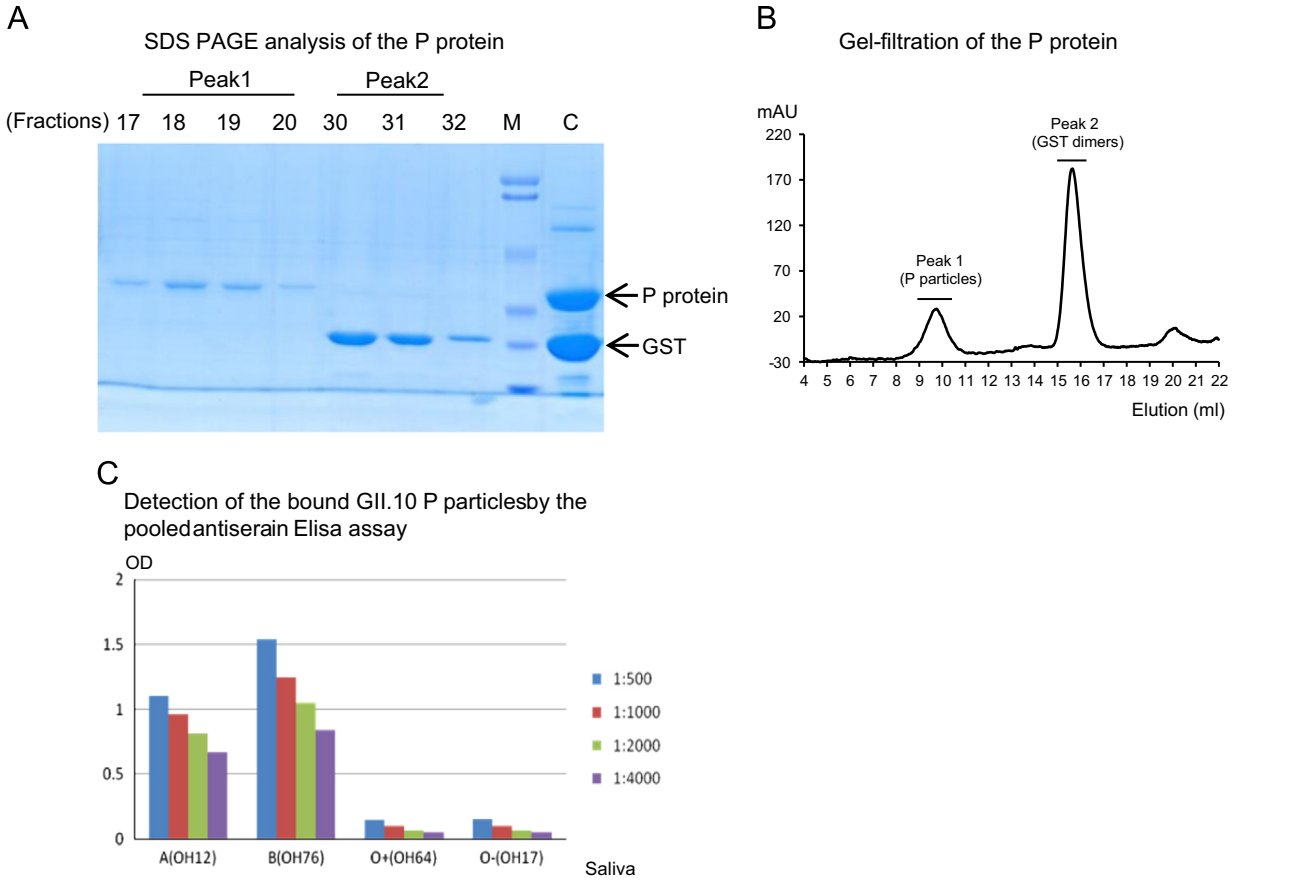

Fig. 1. Production of P particles of Vietnam 026. (A) SDS-PAGE analysis of P proteins. Lane C, the GST–P fusion protein after cleavage by thrombin. Both P protein (~35 kDa) and GST (~26 kDa) are marked with arrows. Fractions of peak 1 and peak 2 were collected from the gel-filtration chromatography (B). Lane M is the prestained protein standards with bands from the top to bottom being 113, 92, 52, 34, 29, and 21 kDa. (B) Gel-filtration analysis of the GST and P proteins using size-exclusion column Superdex 200 (10/300 GL, GE Healthcare Life Sciences). Two major peaks were seen. Peak 1 was near void volume of the column with a molecular weight of ~830 kDa, representing the P particles of the P protein. Peak 2 was corresponding to a molecular weight of ~50 kDa, representing the GST dimers. Four and three fractions of the two peaks were analyzed by SDS-PAGE (A), which revealed P protein and GST, respectively. The gel-filtration columns were calibrated by the Gel Filtration Calibration Kit (GE Healthcare Life Sciences) and the recombinant P particles (830 kDa) of NoV (VA387) and GST dimer of *S. japonicum* (52 kDa). (C) Saliva-based HBGA-binding assays to determine utility and dilution condition of the pooled mouse antisera against the P particles of Vietnam 026. Four saliva samples representing type A (OH12), B (OH76), O secretor (OH64) and nonsecretor (OH17) and four different dilutions (1:500, 1:1000, 1:2000 and 1:4000) of pooled sera were tested. Data were the average value of triplicate experiments. All four saliva samples were Lewis antigen ($Le^a/Le^b/Le^x/Le^y$) positive.