Virology 487 (2016) 296–301

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Structural analysis of bovine norovirus protruding domain

Bishal Kumar Singh [a,b], Anna Koromyslova [a,b], Grant S. Hansman [a,b,*]

[a] Schaller Research Group at the University of Heidelberg and the DKFZ, Heidelberg 69120, Germany
[b] Department of Infectious Diseases, Virology, University of Heidelberg, Heidelberg 69120, Germany

ARTICLE INFO

Article history:
Received 5 August 2015
Returned to author for revisions
1 September 2015
Accepted 21 October 2015
Available online 18 November 2015

Keywords:
Bovine norovirus
Antibodies
Cross-reactivity

ABSTRACT

We determined a structure of a bovine (genogroup III, GIII) norovirus capsid protruding (P) domain using X-ray crystallography. The bovine P domain was reminiscent of other norovirus genogroups (GI, GII, GIV, and GV), but closely matched the human GI P domain. We also identified a monoclonal antibody that was capable of binding the five different (GI–GV) P domains. Our data suggests that genetically diverse noroviruses still contain common epitopes.

Human noroviruses are the leading cause of acute gastroenteritis in humans. Based on the capsid gene (VP1) sequences, these viruses can be classified into at least seven distinct genogroups (GI to GVII). Noroviruses also infect bovine (GIII), mice (GV), cat (GIV), and dog (GIV). Except for murine norovirus, these viruses cannot be grown efficiently in cell culture. Nevertheless, expression of VP1 in insect or mammalian cells can result in the formation of virus-like particles (VLPs) that are antigenically similar to native virions (Hansman et al., 2006).

The X-ray crystal structure of the norovirus VLP identified two domains, shell (S) and protruding (P) domain (Prasad et al., 1999). The S domain forms a scaffold surrounding the viral RNA, whereas the P domain likely contains the determinants for cell attachment and strain diversity. The P domain can be further subdivided into P1 and P2 subdomains and each subdomain likely has unique functions. A number of studies have found cross-reactive antibodies against norovirus genogroups, which indicated shared epitopes and structural elements (Hansman et al., 2006; Batten et al., 2006; Oliver et al., 2006; Parker et al., 2005). Furthermore, serum antibodies against bovine norovirus (GIII) have been detected in humans (Widdowson et al., 2005) and human noroviruses have been identified in bovine stool specimens (Menon et al., 2013), which suggested a possible norovirus zoonosis (Mattison et al., 2007). On the other hand, human noroviruses are known to bind histo-blood group antigens (HBGAs), whereas bovine noroviruses were found to only poorly bind human HBGAs and likely used another type of carbohydrate (Zakhour et al., 2009).

Bovine norovirus was first detected as early as 1976 (Woode and Bridger, 1978), four years after the identification of human norovirus in 1972 (Kapikian et al., 1972). Currently, bovine noroviruses can be grouped into two genotypes, GIII.1 and GIII.2 (Liu et al., 1999; Oliver et al., 2004), where GIII.2 are the most prevalent (Mauroy et al., 2009; Milnes et al., 2007; Park et al., 2007). In this study, we determined the first X-ray crystal structure of a bovine P domain (GIII.2) and compared the structure to other norovirus genogroups (GI.1, GII.4, GIV.2, and GV.1). We found that the overall bovine P dimer was more similar to GI.1 P domain, having a flatten P2 subdomain and smaller extended loops. We also identified a monoclonal antibody (MAb) that was able to bind five different norovirus genogroups.

The GIII.2 bovine norovirus VP1 sequence (Gene Bank accession number EU794907) was aligned with human norovirus GII.10 VP1 sequence (AF504671) in order to determine the P domain region. The P domain of GIII.2 as well as GI.1 (M87661), GII.4 (JX459908), GIV.2 (JF781268), and GV.1 (AEE10027) were expressed and purified as previously described (Hansman et al., 2011). Briefly, the *Escherichia coli*-optimized P domains were cloned into pMal-c2x expression vector and then transformed into *E.coli* BL21 (Invitrogen) cells. Transformed cells were grown in LB medium and expression induced with IPTG (0.75 mM). Cells were harvested and disrupted by sonication. After a series of washing steps, a His-tagged MBP-fusion-P domain protein was eluted from a Ni-NTA column. The fusion protein was digested with HRV-3C protease (Novagen) and the P domain was separated on the Ni-NTA column. The P domain was further purified by size exclusion chromatography with a Superdex-200 column. GIII.2 P domain crystals were grown using a hanging drop method in a 1:1 mixture of protein sample and mother liquor containing 0.2 M zinc acetate, 20% PEG3350 and 0.1 M imidazole (pH 8.0) at 18 °C for 6–45 days.

* Correspondence to: CHS Foundation, University of Heidelberg, and DKFZ, Norovirus Study Group, Im Neuenheimer Feld 242, Heidelberg 69120, Germany. Tel.: +49 (0) 6221 1520.
E-mail address: g.hansman@dkfz.de (G.S. Hansman).

http://dx.doi.org/10.1016/j.virol.2015.10.022
0042-6822/

X-ray diffraction data were collected at the European Synchrotron Radiation Facility, France at beamline ID23-1 and processed with XDS (Kabsch, 1993). The GIII.2 P domain formed crystals in space group C2. Structures were solved using molecular replacement in PHASER (McCoy et al., 2007) using norovirus GI.1 P domain (PDB accession code 2ZL5) as a search model. Structures were refined in multiple rounds of manual model building in COOT (Emsley et al., 2010) with subsequent refinement with PHENIX (Adams et al., 2010). Structures were validated with *Molprobity* (Chen et al., 2010), *Procheck* (Morris et al., 1992), and PDBePISA (Debbink et al., 2014). Atomic coordinates and structure factors of GIII.2 P domain were deposited to the Protein Databank (PDB accession code 5E6T).

The GIII.2 P domain formed rectangular plates that diffracted to 2.2 Å resolution (Table 1). The GIII.2 P1 subdomain comprised of residues 222–274 and 385–507, whereas the P2 subdomain covered residues 275–384 (Fig. 1A). The overall structure of the GIII.2 P domain was highly reminiscent of other noroviruses (Fig. 1B). The P1 subdomain contained a single α-helix and eight β-sheets, while the P2 subdomain comprised of six anti-parallel β-sheets that formed a barrel-like structure. The GIII.2 P1 subdomain closely resembled the other norovirus P1 subdomains (Fig. 1C). The P1 interface-loop, which is involved in HBGA binding to GII noroviruses (Hansman et al., 2011), was also closely similar in size and orientation to the GII norovirus, but different to the other noroviruses (Fig. 1C). The folding and size of β-sheets and outward loops connecting those β-sheets in the GIII.2 P2 subdomain was comparable to the GI.1 P2 subdomain, whereas the GII.4, GIV.2, and GV.1 had larger β-sheets and loops (Fig. 1D). Amino acid sequence alignment of the GI.1 P domain with GII.4, GIII.2, GIV.2 and GV.1 showed that all deletions in the GIII.2 P2 subdomain closely corresponded with the deletions in GI.1 P2 subdomain (Fig. 2A). The GIII.2 P domain dimer had the smallest interface surface (1236.5-Å^2), followed by GI.1 (1542.2-Å^2), GII.4 (1577.3-Å^2), GIV.2 (1770.0-Å^2), and GV.1 (1863.8-Å^2). Taken together, these data indicated that bovine GIII.2 and human GI.1 P domains were relatively similar and some elements were related to the other P domains (see Fig. 2B).

Cross-reactive antibodies between GI and GIII were previously identified and these were mapped to the S domain (Batten et al., 2006; Oliver et al., 2006). However, these antibodies likely detected broken VLPs or linear epitopes (Batten et al., 2006; Oliver et al., 2006). Therefore, in order to identify MAbs that were capable of binding to the GIII.2 P domain, we screened over 30 different MAbs (kindly provided by Doug McAllister, ViroStat, USA) that were produced against different human norovirus VLPs using an antigen ELISA as previously described (Hansman et al., 2007). Briefly, microtiter plates (Maxisorp, Denmark) were coated with 100 µl (10 µg/ml) of P domains in PBS (pH 7.4). Wells were washed and then blocked with skim milk. After washing, 100 µl of serially diluted antiserum was added to each well. The wells were washed and then secondary HRP-conjugated anti-mouse IgG (Sigma) was added to wells. After washing, 100 µl of substrate *o*-phenylenediamine and H_2O_2 was added to wells for 30 min. The reaction was stopped with the addition of 50 µl of 3 N HCl and the absorbance was measured at 490 nm (OD_{490}). All experiments were performed in triplicate. The final OD_{490} = $sample_{mean}$ minus PBS_{mean} (i.e., ~0.05). A cutoff limit was set at $OD_{490} > 0.12$, which was ~2 times the value of the negative control (PBS).

We identified one MAb (termed MAb-4932) that was capable of detecting all five norovirus P domains (Fig. 3A). The other MAbs were either strain specific or cross-reacted with only a few different genogroups (data not shown). MAb-4932 detected GI.1 P domain at a dilution of 800 and the other P domains at a dilution of 400. To confirm the binding, we analyzed the MAb-4932 binding to the P domains using Western blotting (Hansman et al., 2012). At 400 times dilution, MAb-4932 was able to detect all five P domains (Fig. 3B). These results indicated that MAb-4932 was broadly reactive MAb and that the P domain epitope was likely conserved on these P domains. Interestingly, MAb-4932 was developed in mice immunized with a human norovirus GII.4 (Minerva) strain.

Table 1
Data collection and refinement statistics of bovine norovirus P domain structure

PDB ID	5E6T
Data collection	
Space group	C2
Cell dimensions	
a, *b*, *c* (Å)	112.92 63.86 87.56
α, *β*, *γ* (deg)	90 115.06 90
Resolution range (Å)	40.81–2.20 (2.28–2.20)[a]
R_{merge}	14.29 (64.15)[a]
$I/\sigma I$	10.53 (2.79)[a]
Completeness (%)	99.12 (94.02)[a]
Redundancy	5.0 (4.6)[a]
Refinement	
Resolution range (Å)	40.81–2.27
No. of reflections	26,214
R_{work}/R_{free}	19.48/24.12
No. of atoms	4627
Protein	4389
Ligand/ion	8
Water	230
Average *B* factors (Å^2)	
Protein	25.90
Ligand/ion	55.00
Water	27.20
RMSD	
Bond length (Å)	0.005
Bond angle (deg)	0.78

Data set was collected from single crystal.

[a] Values in parentheses are for highest-resolution shell.

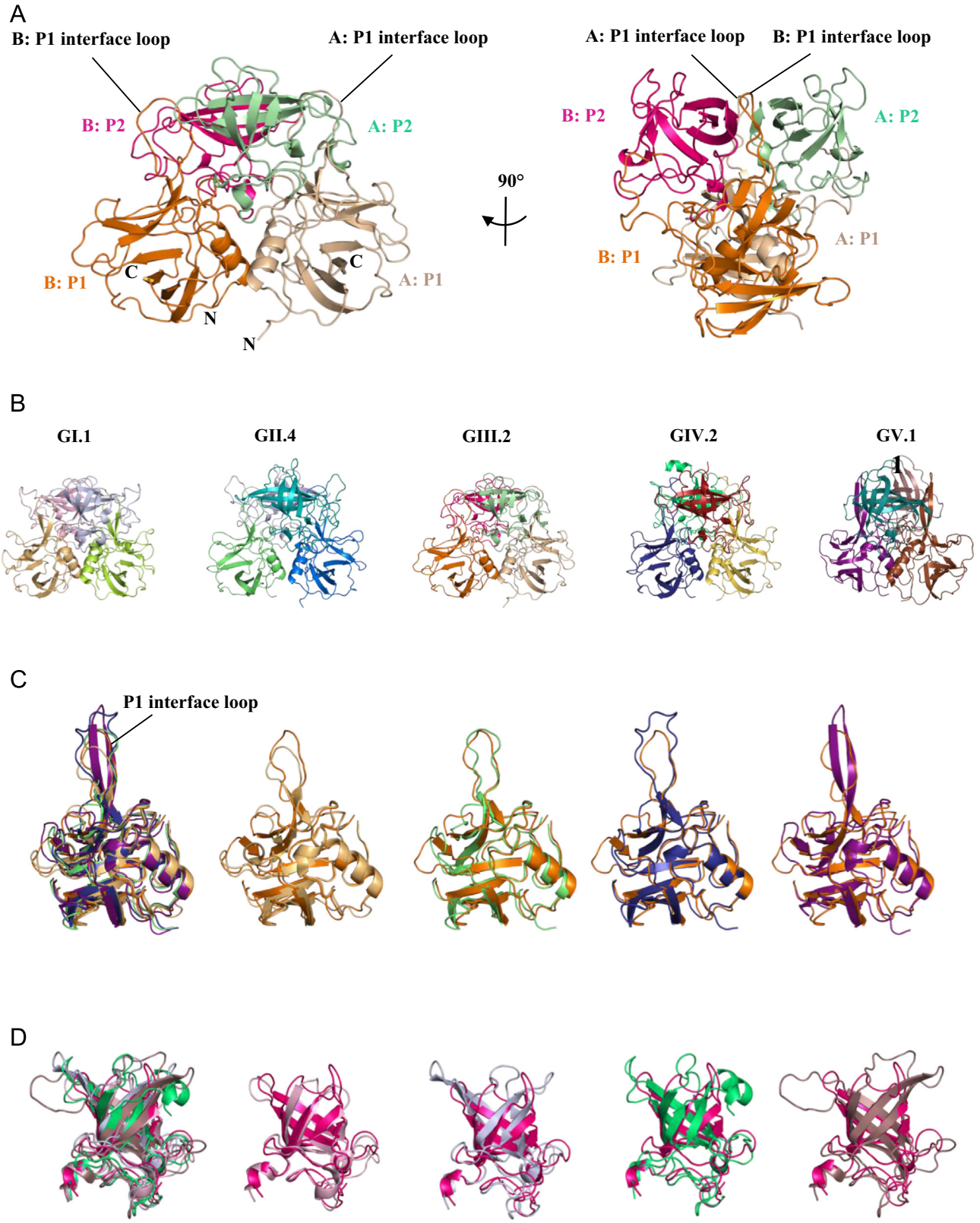

Fig. 1. X-ray crystal structure of unbound GIII.2 P domain. (A) The GIII.2 P domain apo structure contained two dimers per asymmetric unit. The P domain was subdivided into P1 (chain A, wheat and chain B, orange) and P2 (chain A, pale_green and chain B, hot_pink). The GIII.2 P1 interface loop was at the dimer interface and surface exposed. (B) The GI.1 P domain was subdivided into P1 (chain A, limon and chain B, light_orange) and P2 (chain A, blue_white and chain B, light_pink). The GII.4 P domain was subdivided into P1 (chain A, marine and chain B, lime) and P2 (chain A, teal and chain B, blue_white). The GIV.2 P domain was subdivided into P1 (chain A, yellow_orange and chain B, deep_blue) and P2 (chain A, fire_brick and chain B, lime_green). The GV.1 P domain was subdivided into P1 (chain A, brown and chain B, deep_purple) and P2 (chain A, deep_teal and chain B, dirty_violet). The GIII.2 P domain contained similar structural elements as other norovirus P domains, although the overall structure appeared more alike to GI.1 P domain (all drawn to scale). (C) From left to right: superposition of all P1 subdomains (GI.1, GII.4, GIII.2, GIV.2, and GV.1, colored as in B); superposition of GI.1/GIII.2; superposition of GII.4/GIII.2; superposition of GIV.2/GIII.2; and superposition of GV.1/GIII.2. Overall, the P1 subdomains were similar, however, the GIII.2 P1 interface loop closely resembled the GII.4. (D) From left to right: superposition of all P2 subdomains (GI.1, GII.4, GIII.2, GIV.2, and GV.1, colored as in B); superposition of GI.1/GIII.2; superposition of GII.4/GIII.2; superposition of GIV.2/GIII.2; and superposition of GV.1/GIII.2. The GIII.2 P2 subdomain appeared more like the GI.1 P2 subdomain than the other P2 subdomains (C and D were drawn to scale).

A

```
GI.1     RPFTLPNLPLSSLSNSRAPLPISSMGISPDNVQSVQFQNGRCTLDGRLVGTTPVSLSHVAKIRGT---SNGTV----------------- 313
GII.4    K..SV.V.TVEEMT...F.I.LEKLFTG.SSAFV..P......T..V.L...QL.PVNICTF..DVTHIT.SRN---------------- 313
GIII.2   P........VNN..H..VME..AQ.MS.RNFPA..........S.D.L....S.P.DLGAFV.LIAEPGSR.----------------- 309
GIV.2    VS.SV.G.VVEDM....W.AQ.NGLVVRGNEA.V.H.......TE.T.L...TL.INSICGL..LSVSQASVRGAPALTEEMPPLEDEVA 314
GV.1     RMVD..VIQPRLCTHA.W.A.VYGLLVD.SLPSNP.W....VHV..T.L....I.G.W.SCFAAEAA----------------------- 309
              *          *                    ****    * * ***  *

GI.1     ------------------INLTELDGTPFHPF-EGPAPIGFPDLGGCDWHINMTQFGHSSQT----QYDVDT---TPDTFVPHLGSIQAN 360
GII.4    ------YTM----------..ASQNWNDYD.TE.I...L.T..FV.KIQGVLTQTTRTDGS.R-GHKAT.Y.---GSAD.A.K..RV.FE 372
GIII.2   ------------------VE.SQPNQED..AG-SA...F....FSD.SLTFVVASATTVGER-----TVNAR---S.QN.T.A..H.TFD 365
GIV.2    DGAAATYTLARAADTTLWLRVE.P..R.YDI.GDQ...L.T..FTAVIVGTAIRPRTA.GAYLHDA.V.TTP---GDAD.T.ST.NTKIV 404
GV.1     ------YEFQSGTGEVATFTLI.Q..SAYV.G-DRA..L.Y..FS.QLEIEVQ.ETTKTGDKLKVTTFEMILGPT.NADQA.YQ.RVF.S 377
                                        ** * **                                        *  *

GI.1     GIGSGN------YVG-VLSWISP-------PSHPSGSQVDLWKIPNYGSSITEATHLAPSVYPPGFGEVLVFFMSKMPG-------PGAY 438
GII.4    TDTDRD-------FEANQNTKFTPVGVIQDGGTTHRNEPQQ.VL.S.SGRNTHNV....A.A.TFP..Q.L..R.T...----CSGYPNM 451
GIII.2   EEAPAD------LFRAH.R------------NLWDPTEHSF.R..D.RADVLG-SEF....SA..V..T.L...CNV.RL----NGANPN 449
GIV.2    LR.G.S-----GH..QGHY.QFRPIAV---EGGG.RP.YQEYNL.D.AGPTASNHD...P.A.RMP..L.LL.E.D..VWDNGAGAAP.Q 485
GV.1     VTAAASLDLVDGR.RA.PR----------------SIYGFQDT..E.NDGL--LVP...PIG.FLP....LR.RTY.RQIDTA--DAA.E 451
                                             * *          **       ** *  *

GI.1     NLPCLLPQEYISHLASEQAPTVGEAALLHYVDPDTGRNLGEFKAYPDGFLTCVPNGASSGPQQLPINGVFVFVSWVSRFYQLKPV 519
GII.4    D.D......WVQYFYQ.A..AQSDV...RF.N.....V.F.C.LHKS.YV.VAHT--GQHDLVI.P..Y.R.D...NQ..T.A.M 530
GIII.2   PC.......W.T.FV..R.ALQSDV...N..N.N...V.F.A.L.AN....VNLG--A.DQAT..VD.I.K......FY...R.. 507
GIV.2    KIH....N.F.T..FDL...ALA.....R..H..S..T.F...L.RE.YMVVAAP---A.RLNF.LD.Y.R.D....A..I.S.. 565
GV.1     AID.A....FV.WF..NAFTVQS..L..R.RNTL..QL.F...L.NE.YIALSYS--G...LTF.TD.I.EV....P.L...AS. 530
              ** *                 **       *  * * *    *               *  * *   ***   * *
```

B

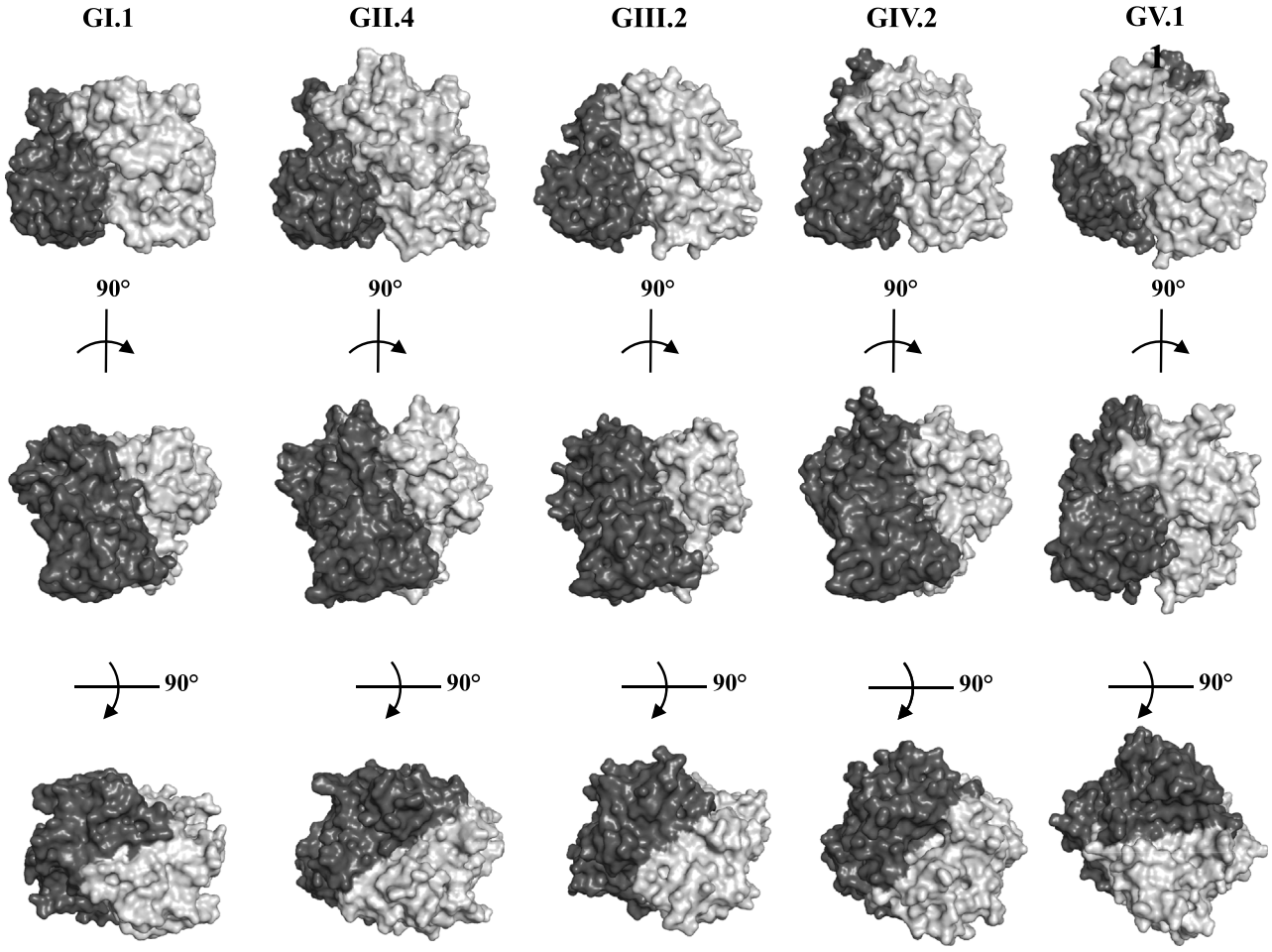

Fig. 2. Amino acid sequence alignment and structural comparisons of the norovirus P domains (A) The P domain amino acid sequences of GI.1, GII.4, GIII.2, GIV.2, and GV.1 noriviruses were aligned using Clustal Omega. The amino acid codes for sequences of P1 and P2 sundomains were shown in colors as depicted in chain A of the P domain dimers in Fig. 1. The P1 interface loop was shaded in light_orange. The asterisks represent conserved amino acids. (B) Surface comparison of the norovirus P domain dimer structures, showing side view, side view rotated 90°, and top view. The GIII.2 P domain dimer was more similar to GI.1 P dimer than the other genogroups. The root-mean-square deviation (calculated in PyMol 1.2r3pre) of the GIII.2 P domain with GI.1, GII.4, GIV.2, and GV.1 were 1.72 Å, 2.24 Å, 2.79 Å, and 3.31 Å, respectively.

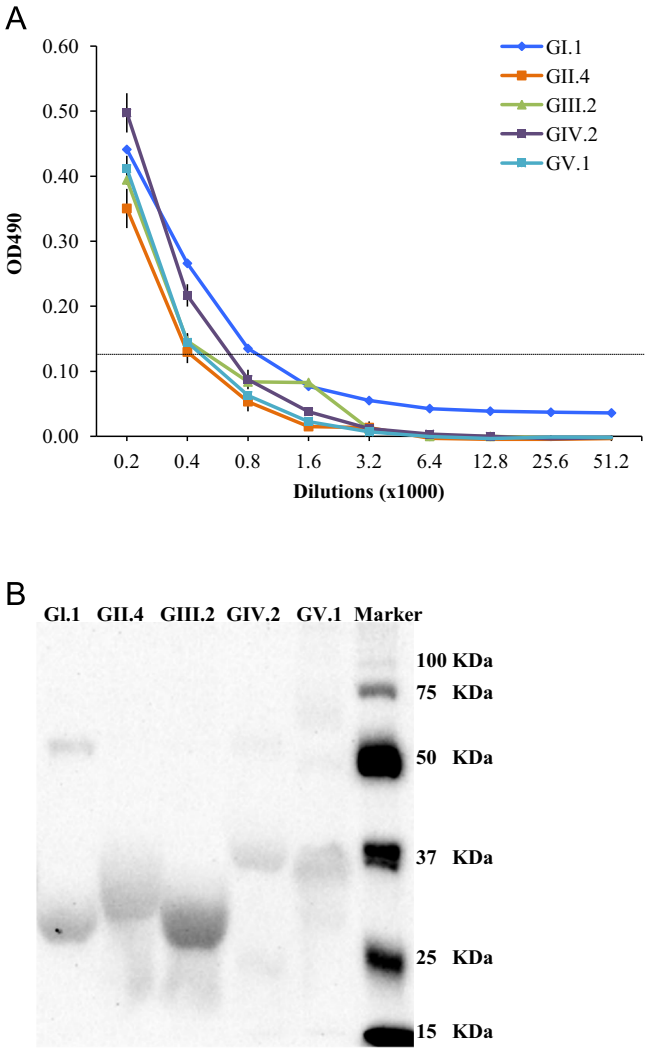

Fig. 3. Norovirus P domain interactions cross-reactivity against MAb-4932. All experiments were performed in triplicate (error bars shown) and the cutoff was at $OD_{490} > 0.12$ (dashed line). Norovirus GI.1, GII.4, GIII.2, GIV.2, and GV.1 P domains were coated on the plate and detected with serially diluted MAb-4932 using a direct ELISA. MAb-4932 detected GI.1 P domains at a dilution of 800 and the other P domains at a dilution of 400. (B) Western blot analysis also showed that the MAb-4932 IgG could detect GI.1, GII.4, GIII.2, GIV.2, and GV.1 P domains (approximately 31, 34, 31, 37, and 36 kDa, respectively).

In summary, we determined the first X-ray crystal structure of a bovine norovirus P domain. We found that the GIII.2 P domain contained comparable structural elements as other norovirus P domains, but the overall structure appeared more similar to GI.1 P domain. We identified a MAb (MAb-4932) that was able to detect five different norovirus genogroups. Taken together, norovirus capsids likely evolved from a common ancestor, but retained certain structural elements and even common epitopes. Further studies are currently in progress in order to determine the binding site of MAb-4932.

Acknowledgments

The funding for this study was provided by the CHS Foundation and the Helmholtz-Chinese Academy of Sciences (HCJRG-202). We acknowledge the European Synchrotron Radiation Facility (ID23-1 and BM30A) for provision of synchrotron radiation facilities and the Protein Crystallization Platform, CellNetworks, Heidelberg for assistance with protein crystallization. We also thank Doug McAllister (ViroStat, USA) for providing the MAb for this study and the helpful discussion.

References

Hansman, G.S., Natori, K., Shirato-Horikoshi, H., Ogawa, S., Oka, T., Katayama, K., Tanaka, T., Miyoshi, T., Sakae, K., Kobayashi, S., Shinohara, M., Uchida, K., Sakurai, N., Shinozaki, K., Okada, M., Seto, Y., Kamata, K., Nagata, N., Tanaka, K., Miyamura, T., Takeda, N., 2006. Genetic and antigenic diversity among noroviruses. J. Gen. Virol. 87, 909–919.

Prasad, B.V., Hardy, M.E., Dokland, T., Bella, J., Rossmann, M.G., Estes, M.K., 1999. X-ray crystallographic structure of the Norwalk virus capsid. Science 286, 287–290.

Batten, C.A., Clarke, I.N., Kempster, S.L., Oliver, S.L., Bridger, J.C., Lambden, P.R., 2006. Characterization of a cross-reactive linear epitope in human genogroup I and bovine genogroup III norovirus capsid proteins. Virology 356, 179–187.

Oliver, S.L., Batten, C.A., Deng, Y., Elschner, M., Otto, P., Charpilienne, A., Clarke, I.N., Bridger, J.C., Lambden, P.R., 2006. Genotype 1 and genotype 2 bovine noroviruses are antigenically distinct but share a cross-reactive epitope with human noroviruses. J. Clin. Microbiol. 44, 992–998.

Parker, T.D., Kitamoto, N., Tanaka, T., Hutson, A.M., Estes, M.K., 2005. Identification of genogroup I and genogroup II broadly reactive epitopes on the norovirus capsid. J. Virol. 79, 7402–7409.

Widdowson, M.A., Rockx, B., Schepp, R., van der Poel, W.H., Vinje, J., van Duynhoven, Y.T., Koopmans, M.P., 2005. Detection of serum antibodies to bovine norovirus in veterinarians and the general population in the Netherlands. J. Med. Virol. 76, 119–128.

Menon, V.K., George, S., Shanti, A.A., Saravanabavan, A., Samuel, P., Ramani, S., Estes, M.K., Kang, G., 2013. Exposure to human and bovine noroviruses in a birth cohort in southern India from 2002 to 2006. J. Clin. Microbiol. 51, 2391–2395.

Mattison, K., Shukla, A., Cook, A., Pollari, F., Friendship, R., Kelton, D., Bidawid, S., Farber, J.M., 2007. Human noroviruses in swine and cattle. Emerg. Infect. Dis. 13, 1184–1188.

Zakhour, M., Ruvoen-Clouet, N., Charpilienne, A., Langpap, B., Poncet, D., Peters, T., Bovin, N., Le Pendu, J., 2009. The alphaGal epitope of the histo-blood group antigen family is a ligand for bovine norovirus Newbury2 expected to prevent cross-species transmission. PLoS Pathog. 5, e1000504.

Woode, G.N., Bridger, J.C., 1978. Isolation of small viruses resembling astroviruses and caliciviruses from acute enteritis of calves. J. Med. Microbiol. 11, 441–452.

Kapikian, A.Z., Wyatt, R.G., Dolin, R., Thornhill, T.S., Kalica, A.R., Chanock, R.M., 1972. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. J. Virol. 10, 1075–1081.

Liu, B.L., Lambden, P.R., Gunther, H., Otto, P., Elschner, M., Clarke, I.N., 1999. Molecular characterization of a bovine enteric calicivirus: relationship to the Norwalk-like viruses. J. Virol. 73, 819–825.

Oliver, S.L., Brown, D.W., Green, J., Bridger, J.C., 2004. A chimeric bovine enteric calicivirus: evidence for genomic recombination in genogroup III of the norovirus genus of the Caliciviridae. Virology 326, 231–239.

Mauroy, A., Scipioni, A., Mathijs, E., Saegerman, C., Mast, J., Bridger, J.C., Ziant, D., Thys, C., Thiry, E., 2009. Epidemiological study of bovine norovirus infection by RT-PCR and a VLP-based antibody ELISA. Vet. Microbiol. 137, 243–251.

Milnes, A.S., Binns, S.H., Oliver, S.L., Bridger, J.C., 2007. Retrospective study of noroviruses in samples of diarrhoea from cattle, using the Veterinary Laboratories Agency's Farmfile database. Vet. Rec. 160, 326–330.

Park, S.J., Lim, G.K., Park, S.I., Kim, H.H., Koh, H.B., Cho, K.O., 2007. Detection and molecular characterization of calf diarrhoea bovine coronaviruses circulating in South Korea during 2004–2005. Zoonoses Public Health 54, 223–230.

Hansman, G.S., Biertumpfel, C., Georgiev, I., McLellan, J.S., Chen, L., Zhou, T., Katayama, K., Kwong, P.D., 2011. Crystal structures of GII.10 and GII.12 norovirus protruding domains in complex with histo-blood group antigens reveal details for a potential site of vulnerability. J. Virol. 85, 6687–6701.

Kabsch, W., 1993. Automatic processing of rotation diffraction data from crystals of initially unknown symmetry and cell constants. J. Appl. Crystallogr. 26, 795–800.

McCoy, A.J., Grosse-Kunstleve, R.W., Adams, P.D., Winn, M.D., Storoni, L.C., Read, R.J., 2007. Phaser crystallographic software. J. Appl. Crystallogr. 40, 658–674.

Emsley, P., Lohkamp, B., Scott, W.G., Cowtan, K., 2010. Features and development of Coot. Acta Crystallogr. D: Biol. Crystallogr. 66, 486–501.

Adams, P.D., Afonine, P.V., Bunkóczi, G., Chen, V.B., Davis, I.W., Echols, N., Headd, J.J., Hung, L.-W., Kapral, G.J., Grosse-Kunstleve, R.W., McCoy, A.J., Moriarty, N.W., Oeffner, R., Read, R.J., Richardson, D.C., Richardson, J.S., Terwilliger, T.C., Zwart, P.H., 2010. Acta Crystallogr. D Biol. Crystallogr. **66**, 213–221.

Chen, V.B., Arendall 3rd, W.B., Headd, J.J., Keedy, D.A., Immormino, R.M., Kapral, G.J., Murray, L.W., Richardson, J.S., Richardson, D.C., 2010. MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr. Sect. D: Biol. Crystallogr. 66, 12–21.

Morris, A.L., MacArthur, M.W., Hutchinson, E.G., Thornton, J.M., 1992. Stereochemical quality of protein structure coordinates. Proteins 12, 345–364.

Debbink, K., Lindesmith, L.C., Donaldson, E.F., Swanstrom, J., Baric, R.S., 2014. Chimeric GII.4 norovirus virus-like-particle-based vaccines induce broadly blocking immune responses. J. Virol. 88, 7256–7266.
Hansman, G.S., Oka, T., Sakon, N., Takeda, N., 2007. Antigenic diversity of human sapoviruses. Emerg. Infect. Dis. 13, 1519–1525.
Hansman, G.S., Taylor, D.W., McLellan, J.S., Smith, T.J., Georgiev, I., Tame, J.R., Park, S. Y., Yamazaki, M., Gondaira, F., Miki, M., Katayama, K., Murata, K., Kwong, P.D., 2012. Structural basis for broad detection of genogroup II noroviruses by a monoclonal antibody that binds to a site occluded in the viral particle. J. Virol. 86, 3635–3646.