The Postfusion Structure of the Heartland Virus Gc Glycoprotein Supports Taxonomic Separation of the Bunyaviral Families *Phenuiviridae* and *Hantaviridae*

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ABSTRACT Heartland virus (HRTV) is an emerging human pathogen that belongs to the newly defined family *Phenuiviridae*, order *Bunyavirales*. Gn and Gc are two viral surface glycoproteins encoded by the M segment and are required for early events during infection. HRTV delivers its genome into the cytoplasm by fusion of the viral envelope and endosomal membranes under low-pH conditions. Here, we describe the crystal structure of HRTV Gc in its postfusion conformation. The structure shows that Gc displays a typical class II fusion protein conformation, and the overall structure is identical to severe fever with thrombocytopenia syndrome virus (SFTSV) Gc, which also belongs to the *Phenuiviridae* family. However, our structural analysis indicates that the hantavirus Gc presents distinct features in the aspects of subdomain orientation, N-linked glycosylation, the interaction pattern between protomers, and the fusion loop conformation. This suggests their family-specific subunit arrangement during the fusogenic process and supports the recent taxonomic revision of bunyaviruses. Our results provide insights into the comprehensive comparison of class II membrane fusion proteins in two bunyavirus families, yielding valuable information for treatments against these human pathogens.

IMPORTANCE HRTV is an insect-borne virus found in America that can infect humans. It belongs to the newly defined family *Phenuiviridae*, order *Bunyavirales*. HRTV contains three single-stranded RNA segments (L, M, and S). The M segment of the virus encodes a polyprotein precursor that is cleaved into two glycoproteins, Gn and Gc. Gc is a fusion protein facilitating virus entry into host cells. Here, we report the crystal structure of the HRTV Gc protein. The structure displays a typical class II fusion protein conformation. Comparison of HRTV Gc with a recently solved structure of another bunyavirus Gc revealed that these Gc structures display a newly defined family specificity, supporting the recent International Committee on Taxonomy of Viruses reclassification of the bunyaviruses. Our results expand the knowledge of bunyavirus fusion proteins and help us to understand bunyavirus characterizations. This study provides useful information to improve protection against and therapies for bunyavirus infections.

KEYWORDS Heartland virus, bunyavirus, glycoprotein C (Gc), postfusion

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Hearthland virus (HRTV) is an emerging virus in the newly defined family *Phenuiviridae*, order *Bunyavirales* (1), which was initially identified from two humans in the United States in 2009 (2). Eight diagnosed human infection cases have been reported in the United States since 2009, and one death occurred in 2013 (3). The virus has also been isolated from nymphal *Amblyomma americanum* ticks, and serosurveillance data show that a number of geographic hosts of *A. americanum* ticks are infected by HRTV (4–6). HRTV is closely related to severe fever with thrombocytopenia syndrome virus (SFTSV), which is associated with hundreds of cases of severe disease in humans in China (7, 8). In the latest taxonomic proposals, SFTSV has been proposed to be reclassified into a novel genus (*Banyangvirus*), making the viral classification more complex.

The order *Bunyavirales* contains nine families, *Feraviridae*, *Fimoviridae*, *Hantaviridae*, *Jonviridae*, *Nairoviridae*, *Peribunyaviridae*, *Phasmaviridae*, *Phenuiviridae*, and *Tospoviridae*, based on a recent reclassification (1). *Phenuiviridae* contains four genera, including *Phasivirus*, *Goukovirus*, *Tenuivirus*, and *Phlebovirus*. HRTV belongs to *Phlebovirus*, *Phenuiviridae*. The genome of *Phlebovirus* consists of three single-stranded RNA segments, designated large (L), medium (M), and small (S) (9). A polyprotein precursor encoded by the HRTV M segment can be cleaved into two glycoproteins, Gn and Gc, which facilitate host cell entry. Gn and Gc likely form a higher-order assembly on the virion envelope (10–13). Gc is proposed to be located at the membrane-proximal portion of the complex according to the combination of electron microscopy (EM) data and crystal structure data of the Rift Valley fever virus (RVFV) Gn, Gc, SFTSV Gn, and Puumala virus (PUUV) Gn (14, 15, 42). Previous structural studies revealed that both hantaviral and phenuiviral Gc fold as class II membrane fusion proteins (14, 16, 17), which is induced by the reduced pH of endocytosis with a conformational change, thereby exposing a hydrophobic fusion peptide or fusion loop. This results in insertion into the endosomal membrane, where the fusion of viral and endosomal membranes occurs, and the viral genetic materials are released for virus replication as host infection starts. Typical class II fusion proteins exhibit a head-to-tail arrangement as homodimers or heterodimers in the prefusion form, while they adopt a trimeric conformation in the postfusion state (18, 19).

To date, two prefusion conformation structures of bunyavirus Gc from RVFV in the *Phenuiviridae* family and Hantaan virus (HTNV) in the *Hantaviridae* family have been solved (14, 17). Although the RVFV Gc ectodomain is monomeric in solution at neutral and acidic pH, two identical dimers can be observed in one asymmetric unit in the crystal. In contrast, the prefusion form of HTNV Gc was obtained by cocrystallization with an antibody fragment, with only one complex in the asymmetric unit. This structure shows that the tip of domain II is partially disordered. Domain III in these two Gc structures displays different orientations toward domain I. Interestingly, the glycosylated Asn1035 in RVFV plays an important role in stabilization of the prefusion dimer. Blocking the glycosylation on this site results in a prehairpin intermediate conformation. In addition, the structures of two hantavirus Gc proteins (PUUV and HTNV) and one phenuivirus Gc (SFTSV) in the postfusion conformation have been determined. The rearrangement of β -strand A₀ that occurs during the fusogenic conformational change of phenuivirus Gc is similar to that of the flavivirus E protein (20, 21). In contrast, this rearrangement among hantaviruses is unusual.

Here, we report the crystal structure of the HRTV Gc ectodomain in a trimeric postfusion conformation. This Gc presents a conformation similar to that of SFTSV in the *Phenuiviridae* family but is distinct from that of HTNV and PUUV in the *Hantaviridae* family. Comparisons between the postfusion forms of Gc between these two families revealed that the domain III orientation, N-linked glycosylation, and interprotomer interaction all display family specificity.

RESULTS

The HRTV Gc ectodomain presents a postfusion trimeric conformation. The recombinant ectodomain of HRTV Gc (residues 567 to 999), lacking the stem region

FIG 1 Overall structure of HRTV Gc in the postfusion conformation. (A) Schematic diagram of HRTV Gc with the crystallized ectodomain colored by domains (domain I in red, domain II in yellow, and domain III in blue). The stem region is colored light gray, and the transmembrane domain (TM) is colored dark gray. The cytoplasmic domain is medium gray. Fusion loops are displayed in orange. Three predicted glycosylation sites are colored green. (B) Structure of HRTV Gc in the postfusion conformation. The trimer is shown with one protomer displayed in the same color scheme as that for panel A in cartoon representation. The other two protomers are indicated in white surface representation. Glycans observed in the structure are shown as green sticks.

proximal to the C-terminal transmembrane region (Fig. 1A), was expressed using the baculovirus system as described in Materials and Methods. The structure of HRTV Gc was refined to 2.1-Å resolution (Table 1). The HRTV Gc crystals were obtained at pH 4.5, and a single trimer of HRTV Gc was observed in the asymmetric unit. The density map was clear for residues 567 to 999, with two internal breaks at loop 582 to 587 and loop 873 to 878. The structure shows that Gc is formed by a central β -sandwich domain (domain I) made of 13 β -strands labeled A_0 through M_0 located in the middle, with domain II and domain III around it. Domain II has an elongated shape with two subdomains, a six-stranded β -sheet and a five-stranded β -sandwich. Strand e in domain II and strand E_0 in domain I connect these two domains. Domain III is an IgC-like module with a seven-stranded β -sandwich. Glycans on N940 in domain III interact with residues in domain I (Fig. 1B).

There are 27 cysteines in the crystallized Gc ectodomain, including 13 disulfide bonds and an unpaired cysteine (Cys621) in domain I. This cysteine pattern is partially conserved across phleboviruses. Interestingly, we solved the structure of another construct missing the first cysteine (residues 568 to 999), which present the same overall conformation, but the disulfide bond between Cys567 and Cys608 is replaced by Cys608-Cys621. This observation is consistent with corresponding cysteine mutation

TABLE 1 Statistics for crystallographic data collection and structure refinement

Parameter	Value(s)
Data collection statistics	
Wavelength (Å)	0.97915
Resolution range (Å)	50.0–2.10 (2.18–2.10)
Space group	P2 ₁ 3
Unit cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	117.88, 117.88, 117.88
α , β , γ (°)	90.00, 90.00, 90.00
No. of unique reflections	32,160 ^a (3,200)
Completeness (%)	100.0 (99.9)
Mean <i>I</i> / σ (<i>I</i>)	23.3 (1.7)
Redundancy	12.8 (6.8)
<i>R</i> _{merge} (%)	11.2 (120.3)
<i>R</i> _{pim} (%)	3.2 (49.5)
CC1/2	0.998 (0.538)
Wilson B factor	40.2
Structure refinement	
Resolution (Å)	50.00–2.10
<i>R</i> _{work} / <i>R</i> _{free} (%)	18.14/23.04
No. of atoms	
Protein	3,170
Ligands	48
Water	206
B factors	
Protein	48.6
Ligands	91.2
Water	49.3
RMSD	
Bond length (Å)	0.008
Bond angle (°)	0.97
Ramachandran plot	
Favored (%)	96.66
Allowed (%)	2.86
Outliers (%)	0.48

^aValues in parentheses are given for the highest-resolution shell.

assay results for SFTSV Gc (C617M), which shown little influence on soluble Gc expression and virus replication (14).

Domain III orientation is bunyavirus family specific. Structural superimposition of each Gc from two members of phenuivirus and hantavirus indicated that Gc proteins in the same genus display similar folds, with root mean square deviations (RMSDs) of 1.010 Å and 1.073 Å, respectively (Fig. 2A and B). In contrast, the HRTV and HTNV Gc display different conformations, with an RMSD of 4.600 Å. Using domain I as a reference, the domain III orientation between these two viruses presents in opposite directions (Fig. 2C). Moreover, the orientation of domain I dramatically changes from the prefusion to postfusion conformation in phenuiviruses, while domain I displays a similar conformation among hantaviruses. The translocation of domain III from the prefusion to postfusion state between these two genera is different as well. Domain III in phenuivirus and hantavirus pivots 75.1° and 132.6°, respectively. In contrast, the hinge of domain II at the domain I/II junction between these two genera is almost the same, displaying 25.9° and 26° shifts, respectively (Fig. 2D and E). Topology diagrams indicate that the two phenuiviruses (SFTSV and HRTV) display similar patterns, with only one 3₁₀ helix (η ₂) difference in domain II (see Fig. S1 in the supplemental material). In contrast, the two hantaviruses (PUUV and HTNV) show more secondary structure element diversity in domains II and III (Fig. S2). Specifically, the PUUV Gc has an extra 3₁₀ helix (η ₂) and a short β -strand (h) in domain II compared to the contents of HTNV Gc. Additionally, there is an α -helix between β -strands j and k in the PUUV Gc domain II, while HTNV has a 3₁₀ helix (η ₂) and a short β -strand (k) instead. In domain III, the HTNV Gc has one β -strand (G) more than the PUUV Gc at its C terminus.

FIG 2 Orientation of domain III displays family specificity in phenuiviruses and hantaviruses. (A) Superimposition of HRTV (blue) and SFTSV (chartreuse) Gc postfusion structures. (B) Superimposition of HTNV (magenta) and PUUV (yellow) Gc postfusion structures. (C) Superimposition of HTNV (magenta) and HRTV (blue) Gc postfusion structures. (D) On the left, the structures of HRTV (blue) postfusion Gc and RVFV (cyan) prefusion Gc were superimposed on domain I. On the right, domain II pivots 25.9° toward the domain I/II junction. The angle between the two domain IIIs rotates 75.1°. (E) Overlapping of HTNV Gc in prefusion (white) and postfusion (magenta) conformation in two views. This shows a 132.5° hinge of reorientation between domain III on the domain I/III junction and 26° of hinge reorientation of domain II about the domain I/II junction. All of the structures are displayed in cartoon representation. Glycans are indicated as green sticks. The RMSDs are labeled below each panel.

Analysis of the topology between phenuiviruses and hantaviruses indicated that β -strand M_0 in domain I is unique in phenuiviruses, which interacts with C_0 in an antiparallel manner. M_0 is the bridging element between domains I and III, which plays an important role in determining the domain III orientation (Fig. 3).

N-linked glycosylation is bunyavirus family specific. Three predicted N-linked glycosylation sites (N857, N918, and N940) are conserved in both HRTV Gc and SFTSV Gc, with one in domain II and two in domain III (Fig. 1A). In both the HRTV and SFTSV Gc structures, only one N-linked glycosylation (N940) in domain III can be observed (Fig. 2A, 3A, and 3B). In the HRTV Gc structure, four glycans (GalNAc β 1,4[Fuc α 1,3Fuc α 1,6]GlcNAc) are linked to N940. Three of them have direct interactions with K625, K627, and E739 in domain I of the same protomer, and one of the glycans interacts with the main chain of K627 through a water molecule (Fig. 4A). In the SFTSV Gc, only one N-GlcNAc can be observed, which interacts with K621 in domain I of the same protomer (Fig. 4B). In contrast, in the structures of two hantavirus Gc proteins, only one glycan linked to N928 (N937 in PUUV) is observed in domain II, which interacts with residues in domain III of the neighboring protomer (Fig. 4C and D). Specifically, in HTNV Gc, the two GlcNAcs linked on N928 in domain II hydrogen bond with the side chains of S1033, T990, and E980 in domain III of the neighboring protomer (Fig. 4C). In PUUV Gc, only the inner GlcNAc molecule of N-glycans in domain II interacts with N999 in domain III of the adjacent protomer (Fig. 4D).

FIG 3 Topology of four bunyaviruses. (A) HRTV; (B) SFTSV; (C) HTNV; (D) PUUV. The color scheme is identical to that described for Fig. 1. Disulfide bonds are labeled as SS and linked with dashes. Glycans are shown as gray hexagons.

Comparison of the interprotomer interactions among class II fusion proteins.

The postfusion Gc trimer exhibits different interprotomer interactions among bunyaviruses. Specifically, the Gc of HRTV and SFTSV presents partial interprotomer interactions (Fig. 5A and B). The Gc proteins of two hantaviruses display cross-protomer interactions (Fig. 5C and D), which is similar to the rubella virus (RuV) E1 protein (Fig. 5H). In contrast, dengue virus (DENV) serotype 1 E protein, tick-borne encephalitis virus (TBEV) E protein, and Semliki Forest virus (SFV) E1 protein have no interprotomer interactions (Fig. 5E, F, G, and J). For phenuivirus Gc (taking HRTV Gc as an example), two hydrogen bond interactions between strand A₀ in domain I and strand M₀ in the adjacent molecule domain I can be observed (Fig. 5I). For hantavirus Gc (taking HTNV Gc as an example), the β -strand A₀ is parallel to β -strand B₀ in the adjacent protomer (Fig. 5K). Furthermore, a similar phenomenon is also observed in RuV E1, which inserts the stem region into the neighboring molecule, thereby being parallel to β -strand I in domain II (Fig. 5L). As it is known that the N terminus plays an important role in the fusogenic conformational change (22), the disulfide bond between C567 and C608, which is conserved in phleboviruses, stabilizes the N terminus (Fig. S3). In contrast, no disulfide bond is formed at the N terminus of hantavirus Gc proteins, and strand A₀ therefore is more flexible for rearrangement (Fig. 3C and Fig. S4). These findings may explain the interchain strand swaps in hantavirus but partial strand swaps in phenuivirus.

Conformationally conserved fusion loop in phenuivirus but not in hantavirus.

The fusion loop, which is responsible for initial insertion into target membrane, is the key element in the fusogenic process. In class II and III fusion proteins, the fusion loop is located at the tip of domain II. However, it is worth noting that Gc in the bunyavirus

FIG 4 N-linked glycosylation sites on four bunyaviruses. (A) HRTV; (B) SFTSV; (C) HTNV; (D) PUUV. Gc structures are shown in cartoon representation. Domains in the same subunit are shown in the same color, while domains in the different subunits are shown in different colors. Glycans are labeled as green sticks. The hydrophobic bonds are indicated as black dashes.

has more than one fusion loop in domain II (Fig. 6). Indeed, three loops (bc loop, cd loop, and ij loop) are obvious at the tip of domain II (Fig. 6A to F). Interestingly, for the phenuiviruses, the conformations of the fusion loops display no difference between the prefusion and postfusion states. There are three disulfide bonds in phlebovirus Gc, one of which links the loop and β -strand, fixing the fusion loop's conformation. In contrast, in hantavirus Gc, two disulfide bonds are located between loops, which is not as stable of a structure as the disulfide bonds in the Gc of HRTV, SFTSV, and RVFV. Specifically, the C745-C780 disulfide bond bridges the ab loop (bc loop in HTNV) and bc loop (cd loop in HTNV), while C775-C905 links the bc loop (cd loop in HTNV) and the ij loop in the postfusion Gc. Moreover, the cd loop and proximal strand b of the bc loop are invisible in the prefusion HTNV Gc, indicating its flexibility (Fig. 6F). The cysteines involved in the fusion loops are conserved in respective families, except for C969 in Toscana virus (TOSV) and C953 in sandfly fever Naples virus (SFNV) (Fig. 6G and H).

DISCUSSION

In this study, we determined the crystal structure of HRTV Gc in its postfusion conformation. The Gc ectodomain of HRTV shares 71% identity to SFTSV Gc. The overall structures between these two Gc proteins are identical. Gel filtration analysis indicated that soluble HRTV Gc is monomeric in solution at both nonacidic pH (8.0) and acid pH (5.0) (see Fig. S5 in the supplemental material), while SFTSV Gc is a mixture of monomeric and a putative trimeric species under pH 5.0 (16). Purified RVFV Gc, PUUV Gc, and HTNV Gc are also monomers in solution both at neutral and acidic pH (14, 17). To date *in vitro*, trimers can only be obtained using liposomes in low-pH solutions (22–25). It will be interesting to further explore why these fusion proteins are monomeric in solution but form dimers or trimers during crystallization.

FIG 5 Comparison of the interprotomer interactions among class II fusion proteins. (A to H) Bottom views of fusion protein trimers from eight different viruses. The three protomers in Gc trimers are shown in white, cyan, and violet, respectively. (A) HRTV; (B) SFTSV; (C) HTNV; (D) PUUV; (E) DENV; (F) TBEV; (G) SFV; (H) RuV. The detailed interactions of the four viruses are shown in panels I to L, and the colors are identical to those described for panels A to H. (I) HRTV; (J) SFV; (K) HTNV; (L) RuV.

Analysis of *N*-linked glycosylation sites in the structure-determined class II fusion proteins indicated that the *N*-linked glycosylation site located at the domain I/III interface plays an important role in determining dimer stability and domain III orientation. Specifically, the *N*-linked glycosylation site on phlebovirus Gc is located in domain III, which interacts with residues in domain I of the same protomer. Blocking *N*-linked glycosylation with tunicamycin during RVFV Gc expression results in an extended form, which seems like a prehairpin intermediate conformation rather than a head-to-tail dimer conformation with *N*-linked glycosylation (14). The *N*-linked glycosylation sites of fusion proteins from alphaviruses (chikungunya virus [CHIKV], SFV, and Sindbis virus [SINV]) and flaviviruses (DENV, Zika virus [ZIKV], West Nile virus [WNV], and TBEV) are located in domain I and also contribute to the stabilization of the prefusion dimer conformation (24–29). In contrast, the *N*-linked glycosylation sites of hantavirus Gc are in domain II, which is distal from the domain I/III junction, thereby increasing the flexibility of domain III during the fusogenic process. However, the structures of SFTSV/HRTV Gc in its prefusion form or RVFV Gc in its postfusion form still are needed to understand phenuivirus Gc rearrangement during fusogenic conformational change.

The fusion process is triggered by low pH. Due to their different protonation states under various pH conditions, histidine residues of the protein are proposed to be the key triggering mechanism leading to fusion (30). Study of the mutation in SFTSV using a reverse genetics system indicates that H747 in domain I and H940 in domain III impair viral replication, suggesting that these two residues are pH sensors. Amino acid alignment of Gc between SFTSV and HRTV shows that H940 is conserved (Fig. S3). A previous study demonstrated that H778, H857, and H1087 (RVFV numbering) are

FIG 6 Comparison of fusion loops in phleboviruses and hantaviruses. The tip of domain II of the postfusion Gc from HRTV (A), SFTSV (B), PUUV (D), and HTNV (E) and the prefusion Gc from RVFV (C) and HTNV (F) are displayed in cartoon representation. The bc loop and cd loop are shown in orange. The disulfide bonds are displayed as green sticks. (G) The amino acid sequence alignment of the fusion loops among 13 phleboviruses. Database sequence accession numbers: HRTV, gb, [AFP33394.1](#); SFTSV, gb, [AGM33042.1](#); RVFV, gb, [DQ380193.1](#); UUKV, gb, [M17417.1](#); SFTV, gb, [NC_015411](#); PPV, gb, [HM566179](#); Palmar virus (PALV), gb, [JQ956380](#); Bhanja virus (BHAV), gb, [JQ956377](#); TOSV, gb, [AMY16462](#); CDUV, gb, [YU_004347992](#); PTV, gb, [ABD92923](#); CHGV, gb, [AEL29641](#); and SFNV, gb, [AEL29667](#). (H) The amino acid sequence alignment of the fusion loops among seven hantaviruses. Database sequence accession numbers: HTNV, gb, [NP_941978.1](#); PUUV, gb, [BAF49040.1](#); Seoul virus (SEOV), gb, [P28729.1](#); Andes virus (ANDV), gb, [AAK14322.1](#); Sin Nombre virus (SNV), gb, [NP_941974.1](#); Adler hantavirus (ADLV), gb, [AIY68300.1](#); and Prospect Hill virus (PHV), gb, [P27315.1](#). Disulfide bonds are labeled in green below the sequences. The secondary structure elements are displayed above the sequences. Strictly conserved and highly similar residues are highlighted in red.

required for virus infectivity (31). H964 (residue H1087 corresponding to RVFV in numbering) is conserved in some phleboviruses (Uukuniemi virus [UUKV], sandfly fever Turkey virus [SFTV], precarious point virus [PPV], TOSV, candiru virus [CDUV], Punta Toro virus [PTV], Chagres virus [CHGV], and SFNV [Fig. S3]), suggesting this histidine is the key pH sensor in phleboviruses. Structural studies show that histidines (H944 in HRTV, H1087 in RVFV, and H940 in SFTSV) that contribute to the stabilization of the structure are located in domain III. A similar histidine (H1038, HTNV numbering) is observed in

hantaviruses as well, and this residue is conserved among hantaviruses (17). However, functional assays have not determined whether H1038 is essential for pH sensing.

The distinct features of Gc from either phenuiviruses or hantaviruses indicate newly defined family specificity. This calls for further structural work on other family members, which should be addressed in the near future.

MATERIALS AND METHODS

Protein expression and purification. The HRTV Gc ectodomain (residues 567 to 999; GenBank accession number [AFP33394](#)) was prepared using the baculovirus expression system as previously reported (32–35). Specifically, codon-optimized synthetic DNA encoding the HRTV Gc ectodomain was cloned into the pFastBac1 vector (Invitrogen) in frame with an amino-terminal gp67 signal peptide for secretion and a 6×His tag at the C terminus for purification. Transfection and virus amplification were performed according to the Bac-to-Bac baculovirus expression system manual (Invitrogen). Gc protein was produced by infecting Hi5 cells for 48 h. Soluble Gc was purified from cell supernatants by metal affinity chromatography using a 5-ml HisTrap HP column (GE Healthcare) and then further purified using a Superdex 200 10/300 GL column (GE Healthcare) in 20 mM Tris (pH 8.0) and 50 mM NaCl, and the protein fractions were concentrated to 5 mg/ml.

Crystallization and structure determination. Crystallization conditions of HRTV Gc were screened by the sitting-drop vapor diffusion method at 18°C, with 1 μ l of 5 mg/ml Gc mixed with 1 μ l of reservoir solution. Gc crystals were obtained in 6% (vol/vol) 2-propanol, 0.1 M sodium acetate trihydrate (pH 4.5), and 26% (vol/vol) polyethylene glycol monomethyl ether 550. Crystals were frozen in liquid nitrogen in reservoir solution supplemented with 20% glycerol (vol/vol) as a cryoprotectant. Data were collected at 100 K at the Shanghai Synchrotron Radiation Facility beamline BL17U. All data were processed with HKL2000 software (36). The structure of Gc was solved by molecular replacement using the SFTSV Gc molecule (Protein Data Bank [PDB] code [5G47](#)) as a search model with Phaser in the CCP4 program suite (37). Initial restrained rigid-body refinement and manual model building were performed with REFMAC5 (38) and COOT (39), respectively. Further refinement was performed with Phenix (40). The stereochemical quality of the final model was assessed with the program PROCHECK (41). Figures were prepared by using PyMOL.

Accession number(s). Data were deposited in PDB under code [5YOW](#).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/JVI.01558-17>.

SUPPLEMENTAL FILE 1, PDF file, 2.1 MB.

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We have no conflicts of interest to declare.

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