Structure of Seneca Valley Virus-001: An Oncolytic Picornavirus Representing a New Genus

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SUMMARY

The crystal structure of Seneca Valley Virus-001 (SVV-001), the representative member of a new genus, Senecavirus, is reported at 2.3Å resolution. SVV-001 is the first naturally occurring nonpathogenic picornavirus shown to mediate selective cytoxicity towards tumor cells with neuroendocrine cancer features. The nonsegmented (+) ssRNA genome of SVV-001 shares closest sequence similarity with the genomes of the members of Cardiovirus. The overall tertiary structure of VP1-VP4 subunits is conserved with the exception of loops, especially those of VP1 that show large deviations relative to the members of the cardioviruses. The surface loops of VP1 and VP2 are predicted to mediate cell tropism of SVV-001. In addition, the organization of the packaged nucleic acid density indicates that certain regions of VP2 and VP4 interact closely with the packaged nucleic acid.

RESULTS AND DISCUSSION

Structure of SVV-001

The protomer of SVV-001 is composed of four different subunits, VP1, VP2, VP3, and VP4, of lengths 263, 284, 239, and 72 residues, respectively. The overall folds of the subunits (Figure 1) are very similar to the corresponding proteins in other viruses from the Picornaviridae family. Similar to other cardioviruses (Grant et al., 1992; Krishnaswamy and Rossmann, 1990; Luo et al., 1987, 1992), VP1 of SVV-001 possesses a hydrophobic pocket without a pocket factor. However, the entrance to the hydrophobic cleft in SVV-001 is almost completely sealed off by residues W140, F180, W184, D213, and W214 from the VP1 subunit, whereas in the cardioviruses and rhinoviruses (Hadfield et al., 1997; Kim et al., 1989; Verdaguer et al., 2000; Zhao et al., 1996) the entrance is narrow and wide, respectively.

The reasons for the variant cell tropism of picornaviruses are owing to the composition, location, length, and disposition of surface-exposed loops of the capsid proteins, which are typical of individual viruses in Picornaviridae (Rossmann et al., 1985). In SVV-001, BC loop (residues 48–72), loop II (CD loop, residues 92–107), and FMDV loop (GH loop, residues 183–213) of VP1, the “puff” (EF loop, residues 170–198) of VP2, and the “knob” (residues 55–71) of the VP3 subunit are of particular importance (Figure 1) as they are more prominent in length when compared with other members of Picornaviridae (Filman et al., 1998; Fry et al., 1998; Yanagi et al., 2006). A new member to join this group is SVV-001, the representative member of a new genus, Senecavirus, is reported at 2.3Å resolution.
Figure 1. Protomer Structure and the Quality of the Electron Density Map of SVV-001

(A and B) Ribbon diagrams of SVV-001 protomer, (A) front view and (B) a side view, highlighting the different subunits and the prominent surface loops. VP1, VP2, VP3, and VP4 subunits are shown in blue, green, red, and yellow, respectively.

(C) Stereo diagram showing the quality of the NCS-averaged electron density map at 2.3 Å resolution of residues 115–120 in the VP1 subunit, contoured at 1.2σ.
et al., 2003; Grant et al., 1992; Hadfield et al., 1997; Hogle et al., 1985; Kim et al., 1989; Krishnaswamy and Rossmann, 1990; Logan et al., 1993; Luo et al., 1992; Muckelbauer et al., 1995; Rossmann et al., 1985). On the contrary, loop I (CD loop, residues 80–85 of VP1) in SVV-001 is shorter and concealed by the loops BC and II of VP1. Apart from the knob of VP3 subunit, the other loops of SVV-001 are structurally well ordered.

The capsid of SVV-001 has a maximum diameter of 325 Å with a contiguous shell thickness of 25 Å and surface unevenness (Figure 2A). The highest elevation (325 Å in diameter) occurs close to the fivefold axis and is formed by the stacking of three loops, two from VP1 (loops BC and II) and the third from VP2 (the puff). The knob of VP3 stands alone closest to the threefold axis. The accessible surface area of the residues of the various loops of the three subunits, as calculated using VIPERdb (Shepherd et al., 2006), suggests that the loops II of VP1 and the knob of VP3 have the highest effective solvent-accessible surface area (SASA) of ~7000 Å². The puff of the VP2 subunit has an effective SASA of ~6000 Å². As opposed to members of the rhinovirus family that display more prominent VP2 loops and smaller VP1 loops, members of cardiovirus have prominent loops of both VP1 and VP2. However, the lengths and disposition of these loops in SVV-001 and the other cardioviruses leads to varied surface features and the filling up of the canyon. Instead of the canyon, SVV-001 and the members of Cardioviridae show a sharp pit.

Analysis of the RMSDs of the main chain Cα atoms of the protomer of SVV-001 with other representative members of Picornaviridae shows a maximum (1.4 Å, number of aligned residues ~500) and minimum (1.2 Å, number of aligned residues ~600) deviation with the members of rhinovirus (Hadfield et al., 1997; Kim et al., 1989; Verdaguer et al., 2000; Zhao et al., 1996) and cardiovirus (Grant et al., 1992; Krishnaswamy and Rossmann, 1990; Luo et al., 1992), respectively. In accordance with the structural similarity, SVV-001 shows maximum (~42%) and minimum (~30%) sequence identity with mengo encephalomyocarditis virus (MEV) and human rhinovirus (HRV) 16, respectively. Figures 2C–E show the superposition of the subunits of MEV onto corresponding subunits of SVV-001. Individually, the VP1 and VP2 subunits of SVV-001 show maximum and minimum structural variation, respectively, with the other members of Picornaviridae.

**Nucleic Acid Arrangement**

A low-resolution (~20 Å) map of SVV-001 showed the arrangement of nucleic acid inside the capsid shell, visualized using X-ray crystallography for the first time for any picornavirus. The bulk nucleic acid organization in SVV-001 seems to be continuously in contact with the inner surface of the capsid, with more prominent contacts near the icosahedral twofold axes beneath two VP2 subunits (Figure 3). There is an innermost compact sphere of density at the center of the viral capsid surrounded by a few discontinuous isolated densities. Closest to the capsid layer is a more continuous shell of RNA density that interacts with residues 41–43, 55–57 of VP2 and 63, 64 of VP4 subunits and could potentially play a role as a scaffold for capsid assembly and architecture. It is possible that similar nucleic acid organization may also be present in other picornaviruses.

**Putative Receptors of SVV-001**

Though there are receptors known for many picornaviruses (Rossmann et al., 2002), receptor(s) for Senecaviruses are yet to be determined. Because SVV-001 is known to target cells with neuroendocrine tumor features, it is possible that the cell tropism of SVV-001 might be governed by binding to receptors expressed on such tumor cells. A few that have been suggested include markers such as gastrin-releasing peptide receptors,
synaptophysin, neuron-specific enolase, and CD56 (Wadhwa et al., 2007). However, no structural evidence could be established for the binding of SVV-001 to any of these receptors. Nevertheless, the outer capsid features of SVV-001 were analyzed to gain insights into other possible receptors to which it might bind (Figures 4A and 4B). Of these were the \( \alpha_v \beta_4 \) integrins, which are recognized by the arginine-glycine-aspartic acid (RGD) motif in foot-and-mouth disease virus (FMDV) (Logan et al., 1993). It is known that the lysine-glycine-aspartic acid (KGD) motif mimics RGD in many cases (Reiss et al., 2006; Lu et al., 2006). Although the motif KGD does not occur on the surface of SVV-001 capsids, its reverse motif, DGK (residues 146–148), is found in the VP2 subunit of SVV-001. The main chain conformation of RGD bound to integrin molecule in a RGD-integrin complex (Xiong et al., 2002) matches well with the main chain conformation of the DGK motif of SVV-001 (Figure 4C). Furthermore, the DGK motif is exposed on the surface of SVV-001 and is located in the canyon formed by the VP1/2 loops and the puff of VP2. So it is likely that the DGK motif might play an important role in recognizing integrin receptors on the cell surfaces. In addition to the DGK motif, another integrin binding motif, LDV (residues 141–143 of VP2), also exists on the surface of SVV-001 capsids. This motif is known to bind to specific \( \alpha_v \beta_4 \) integrins expressed on small-cell lung cancer cells (Tselepis et al., 1997). However, the LDV motif is not exposed on the surface and lies in a depression that is immediately next to the puff of VP2 subunit and is less accessible (Figure 4D). However, it is possible that local conformational changes could expose this motif and aid in receptor binding. Because DGK and LDV motifs are structurally proximal (Figure 4B), they could be jointly involved in binding to integrins on the cell surface.

Another receptor that SVV-001 could bind to is low-density lipoprotein receptor (LDLR). Essentially the N-terminal extracellular region of LDLR is the one that is recognized by viruses. The structure of HRV2 complexed with a fragment of LDLR (Verdaguer et al., 2004) suggests that three residues interact with the receptor, of which K224 of VP1 is most crucial and conserved in all minor group members (e.g., HRV1a). The structural superposition of SVV-001 onto HRV2-LDLR showed that a highly exposed lysine (K227) of VP1 in SVV-001 nearly overlaps with the conserved lysine K224 of HRV2, which is known to be involved in crucial interactions with the LDLR. Except for this lysine, the other proximal residues are variable among the members of minor group rhinoviruses that bind to the LDLR. The K227 of SVV-001 is exposed with an effective SASA of 4500 \( \AA^2 \) and could play a role in binding to LDLR, suggesting that the LDLR is another plausible receptor for SVV-001 (Figure 4E).

Additionally, structural comparison of SVV-001 with MEV (Krishnaswamy and Rossmann, 1990; Luo et al., 1987) shows that there is no corresponding lysine for K227 in the MEV structure. The loop (HI), which harbors K227 in SVV-001, is much shorter in MEV and takes a different path. Although the SVV-001 structure is closest to MEV, it resembles rather well locally with the LDL-binding region of HRV2. However, the LDV motif seen in SVV-001 is also present in MEV, but absent in HRV2. The LDV motif in MEV is surface accessible; however, the involvement of this motif in receptor binding has not been investigated for MEV. The DGK motif seen in SVV-001 is not present in MEV.

EXPERIMENTAL PROCEDURES

The SVV-001 was crystallized in a R3 space group with unit cell dimensions \( a = b = 311.5 \AA, c = 1526.4 \AA, \alpha = \beta = 90^\circ, \gamma = 120^\circ \), and its structure was determined at 2.3 Å resolution by molecular replacement using MEV capsid as the model (Venkataraman et al., 2008). The unit cell of SVV-001 is unusual because of a long c axis (1526 Å) that is nearly 4.9 times the lengths of a or b axis (311 Å) and hence accommodates six virus particles. Two sets of 20 mers contributed by two distinct virus particles located at (0, 0, 0) and (0,033, 0.666, 0.333).
Figure 4. Locations of Putative Receptor Binding Sites on SVV-001

(A) Positions of major receptor binding motifs on the surface of SVV-001. Sites 1 and 2 indicate the locations of the DGK and the LDV motif; K227 is site 3. The LDV motif (site 2) is hidden under the DGK motif (site 1) and is indicated by an arrow. The icosahedral asymmetric unit comprising VP1-4 is indicated by a trapezoid.

(B) Enlarged view of the icosahedral asymmetric unit oriented to highlight the positions of DGK, LDV, and K227.

(C) The superposition of DGK motif (gold) on the RGD motif (cyan).

(D) Location of the LDV motif, shown as purple surface and stick diagram, in the VP2 subunit.

(E) Structural superposition of loop containing K227 (VP1) of SVV-001 (pink) onto LDL binding loop of HRV2. The structural proximity of K227 of SVV-001 with the critical residue, K224 of HRV2 in the HRV2-LDL receptor (baize-colored surface) complex, indicates that it might play a similar role in binding to the LDL receptor in SVV-001.

0.166) occupy the crystallographic asymmetric unit. The orientation of the two distinct particles was determined using the program GLRF (Tong and Rossmann, 1990), and the particle positions were determined using the program Phaser (Read, 2001; Storoni et al., 2004). The initial phase extension and NCS averaging using RAVE (Kleywegt and Jones, 1994) and CCP4 suite of programs (CCP4, 1994) resulted in readily interpretable maps at 2.3 Å with a final real-space R factor of 0.23 and correlation coefficient of 0.93.

The final model was built manually into the electron density map contoured at 1.2 σ using the program O (Jones et al., 1991). The structure was refined using the program CNS (Brunger et al., 1998) imposing (strict) 40-fold noncrystallographic symmetry (NCS) constraints. Water molecules were picked initially using the program CNS and later manually from once averaged Fo-Fc map contoured at 2.5 σ.

A low-resolution (100–20 Å) vector difference map was calculated by subtracting model amplitude and phases from the Fobs and phase-refined phases to visualize the bulk nucleic acid density. The final refinement statistics are shown in Table 1.

Model quality was analyzed using PROCHECK (Laskowski et al., 1993), and Stride (Heinig and Frishman, 2004) was used for the assignment of secondary structures. TOP2d from CCP4 suite of programs and ALIGN (Cohen, 1997) were used for structure alignment. PyMOL (http://www.pymol.org), Chimera (Pettersen et al., 2004), and VMD (Humphrey et al., 1996) were used for structure visualization, analysis, and generation of figures. VIPERdb analysis (Shepherd et al., 2008) was employed to estimate buried and accessible surface areas and association energies. Intra/intersubunit contacts, average B, and individual accessible surface area were calculated using CCP4 suite of programs (CCP4, 1994).

**ACCESSION NUMBERS**

Coordinates have been deposited in the PDB with accession code 3CJI.

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