

Tellina virus 1 VP4 peptidase

DATABANKS

MEROPS name: Tellina virus 1 VP4 peptidase

MEROPS classification: clan [SJ](#), family [S69](#), peptidase [S69.001](#)

Tertiary structure: [Available](#)

Species distribution: known only from *Tellina virus 1*

Reference sequence from: *Tellina virus 1*

Name and History

While examining a population of marine bivalve mollusks *Tellina tenuis* it was noticed that the shells of a subpopulation of these clams were markedly thinner and chalkier [1]. Also, the digestive glands of these mollusks were pale yellow instead of the usual dark brown. Further examination revealed a high proportion of the cells in the digestive gland, which produce the shell precursor material, were necrotic and harbored membrane-bound inclusion bodies with icosahedral viral particles of ~70 nm in diameter. Similar viral particles were absent in the apparently healthy population. The virus showed similar morphological and physicochemical characteristics to *Infectious Pancreatic Necrosis Virus* (IPNV), an aquabirnavirus that infects salmonid fish [2,3]. Accordingly, it was classified as a member of the Birnaviridae family and named *Tellina Virus 1* (TV-1) to differentiate it from *Tellina Virus 2* (TV-2) [4–6].

Birnaviruses infect vertebrates, mollusks, insects and rotifers [7]. These viruses are categorized into three genera according to the hosts they infect: avibirnavirus, aquabirnavirus and entomobirnavirus infecting birds, aquatic organism and insects respectively. A well-studied example of avibirnavirus is *Infectious Bursal Disease Virus* (IBDV) that destroys lymphoid cells in the bursa of Fabricius in chickens [8]. Aquabirnavirus *Infectious Pancreatic Necrosis Virus* (IPNV) causes disease in salmonid fish. Entomobirnavirus *Drosophila X Virus* (DXV) causes sensitivity to anoxia in flies [9]. Birnaviruses carry a bi-segmented (Segment A and B) double-stranded RNA genome enclosed in an icosahedral capsid ($T = 13$) of around 60–70 nm in diameter [4,10–12]. The largest open reading frame in segment A encodes a polyprotein that has the conserved arrangement of NH₂-pVP2-VP4-VP3-COOH (Figure 780.1) [6,13]. An exception is found in TV-1 and *Blotched Snakehead Virus* (BSNV) where gene X is inserted between pVP2 and VP4. The self-encoded VP4 protease cleaves the polyprotein into its constituent components. Capsid precursor protein pVP2 is further processed to yield VP2 and additional peptides [14]. VP3 associates with the genome to form a ribonucleoprotein complex [15]. In IPNV and IBDV, a smaller open reading in segment A codes for VP5, a protein that regulates host apoptosis [6,16,17]. Birnaviruses also encode a viral RNA polymerase (VP1) in segment B of the genome [18].

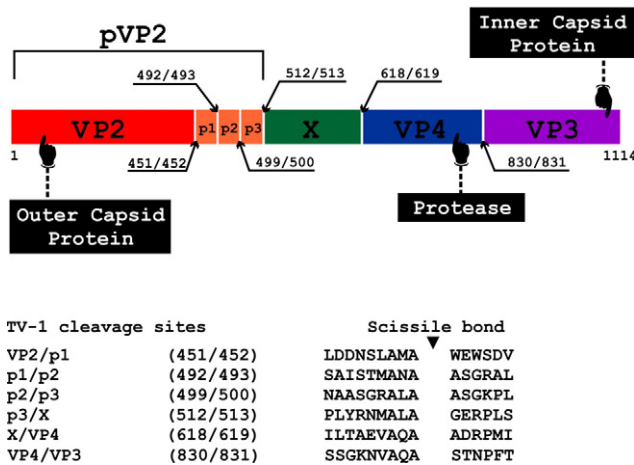


FIGURE 780.1 The TV-1 polyprotein cleavage sites. (A) The genomic arrangement of TV-1 segment A. The arrows indicate the cleavage sites with the P1 and P1' residue numbers listed; (B) A list of known TV-1 polyprotein cleavage sites [6].

Activity and Specificity

Nobiron *et al.* [6] identified the TV-1 cleavages at the X↓VP4 and VP4↓VP3 junctions by first expressing regions of the polyprotein in *Escherichia coli*, then subjecting the cleavage products to N-terminal sequencing (Figure 780.1). The pVP2↓X junction and the secondary cleavage sites within pVP2 were identified by mass spectrometry analysis of the virions. The primary cleavage sites are located at the C-terminal end of residues 512, 618, and 830 within the polyprotein (Figure 780.1) [6]. Cleavages at these sites yield pVP2, peptide X, VP4, and VP3 [6,19]. Additional processing occurs at the C-terminal end of residues 451, 492, and 499 within pVP2. The identified cleavage sites can be described by the consensus sequence Ala-Xaa-Ala↓. Consistent with this specificity, the S1 and S3 binding pockets of the VP4 protease are shallow and hydrophobic [20]. In TV-1 [6], MABV [21], IPNV [22], BSNV [13], and IBDV [23], an alanine is found at the P1 position of the primary cleavage sites. However, the residues upstream of the P1 position are less conserved among these birnaviruses. The crystal structure of TV-1 VP4 solved by Chung & Paetzel captured an intramolecular (*cis*) acyl-enzyme complex at the VP4/VP3 junction (Figure 780.2). The last three residues of the protein construct used for the TV-1 VP4 structure correspond to the P3-P1 positions at the VP4/VP3 junction. These residues interact with the substrate binding groove by forming antiparallel β-sheet style hydrogen bonding interactions on one side of the groove and parallel β-sheet style hydrogen bonding interactions on the other side of the groove (Figure 780.3). A water molecule (W2) serves as a hydrogen bond linker between Asp653 of the binding groove and Val827 (P4) of the substrate/product. In TV-1 VP4 protease, a Phe is found at position 736 while a

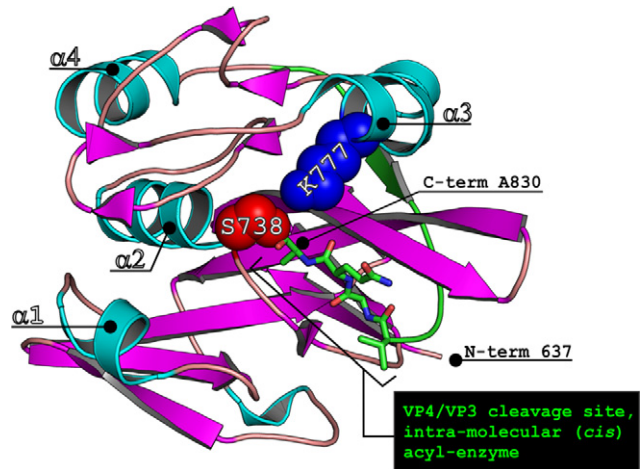


FIGURE 780.2 The protein fold of TV-1 VP4 protease. TV-1 VP4 protease contains 4 α -helices (cyan, α 1–4), 16 β -strands (magenta), and two 3_{10} helices (shown in cyan) [20]. The nucleophile, Ser738, is shown as red spheres and the general base, Lys777, as blue spheres. The C-terminal residues (Val814-Ala830) of VP4 are shown in green with the P1-P4 shown as sticks (carbons, green; nitrogen, blue; and oxygen, red). The last residue (Ala830) forms an intramolecular (*cis*) acyl-enzyme with the serine nucleophile (Ser738, red spheres).

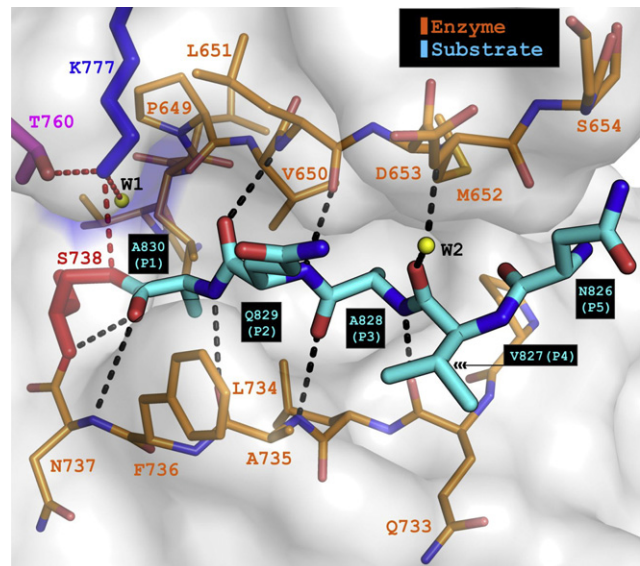


FIGURE 780.3 Enzyme-substrate interactions revealed in the intramolecular (*cis*) acyl-enzyme complex of TV-1 VP4 protease. The O⁻ of the serine nucleophile (Ser738, red) forms an ester bond with the carbonyl carbon of Ala830 (cyan), the last residue of VP4 and the P1 site at the VP4/VP3 junction [20]. The C-terminal residues (826–830) form a hydrogen bonding network (dashed black lines) with the substrate binding groove (orange). The proposed deacylating water is labelled as W1. A water molecule (W2) acts as a hydrogen bond linker between Val827 and Asp653. Hydrogen bonds involving the catalytic residues are shown as red dashed lines. The general base (Lys777) is shown in blue and a conserved active site threonine (Thr760) is shown in magenta. The background is the semitransparent molecular surface of the VP4 molecule.

Gly is found at the comparable position in the IPNV, IBDV, DXV, MABV, BSNV and Rotifer Birnavirus. The residue at this position forms part of the S1 binding pocket in TV-1 and IPNV VP4 proteases.

Structural Chemistry

In 2006, Feldman *et al.* [24] elucidated the first birnavirus VP4 protease structure from BSNV. This structure was solved with a native active site that revealed the serine/lysine catalytic dyad but without anything bound within the substrate-binding groove. Two IPNV VP4 structures were solved in 2007 by Lee *et al.* [25] revealing an intermolecular (*trans*) acyl-enzyme complex and a structure with an empty substrate-binding groove. The acyl-enzyme complex was formed between the C-terminal carbonyl carbon of an adjacent VP4 molecule (the product of an internal VP4 cleavage site) and the nucleophilic serine O γ of VP4 with the general base mutated to a lysine. The crystal structure of TV-1 VP4 was solved by Chung & Paetzel in 2011 [20] at 2.1 Å resolution. It revealed an intramolecular (*cis*) acyl-enzyme complex at the VP4/VP3 junction. This structure, like the IPNV intermolecular complex structure, reveals the cleavage site specificity residues (P3–P1) within the substrate specificity pockets (S3, S1), but in addition the TV-1 VP4 shows directly how the C-terminus of VP4 is able to fold around itself to cleave at the VP3/VP4 junction. This acyl-enzyme complex was formed within a native active site. TV-1 VP4 protease is encoded as part of the 1114 residue-long segment A polyprotein and is composed of 212 amino acids (residues 619–830) with a calculated size of 22 450 Da and a theoretical pI of 9.8. TV-1 VP4 has an α/β -fold (Figure 780.2) with 16 β -strands, four α -helices and two 3_{10} helices. Beta-strands 1–9, 15 and 16 come together to form two β -sheets that house the substrate-binding groove and specificity pockets. This region also has one 3_{10} helix and one α -helix. Above this β -sheet platform is a parallel β -sheet made up of three short strands (β 10, 13, and 14) flanked by three α -helices (α 2–4) and a β -hairpin (β 11, 12). The C-terminal residues (814–830) pass under a protruding loop before entering the substrate-binding groove. Using site-directed mutagenesis, serine738 has been identified as the nucleophile and Lys777 as the general base in the TV-1 VP4 protease mechanism [6]. The position and the orientation of Ser738 and Lys777 in the crystal structure are consistent with the findings of the mutagenesis study [20]. This serine/lysine catalytic dyad mechanism is utilized by all birnavirus VP4s (Chapter 779). In the active site, the O γ of Ser738 forms an ester bond with the carbonyl carbon of Ala830, the P1 site on the substrate, reaffirming Ser738 as the nucleophile. The electron density within the active site clearly shows the trigonal planar geometry for the acyl-enzyme ester linkage. The O γ of the conserved

Thr760 is within hydrogen bonding distance to the N ζ of the general base Lys777. It is likely Thr760 helps to coordinate Lys777 into the correct position to abstract a proton from the hydroxyl of the nucleophile (Ser738). The ester carbonyl oxygen of the P1 residue (Ala830) points into an oxyanion hole made up of the main chain amide nitrogens of Ser738 and Asn737. A water molecule (W1) lies behind the catalytic dyad and is at approximately the right position to serve as a deacylating water with a Bürgi angle of 155° (Figure 780.3) [26].

Preparation

The DNA region encoding TV-1 VP4 protease was cloned into vector pET28b⁺ with an N-terminal 6x histidine-tag [27]. The calculated molecular mass for this tagged construct is ~25 kDa. The construct was expressed in *E. coli* strain Tuner (DE3). Upon purification by nickel-nitrilotriacetic acid (Ni-NTA) metal affinity chromatography followed by size-exclusion chromatography (SEC) the protein appeared to be multimeric as it eluted in the void volume during SEC. To produce monomeric protein, the void volume fractions were pooled and applied to a SP Sepharose FF cation-exchange column. The cation-exchange fractions containing VP4 were pooled and loaded onto the SEC column for a second time. The protein then eluted at a volume consistent with a size of ~25 kDa (monomer). The purified VP4 was subjected to limited-proteolysis by chymotrypsin prior to crystallization trials. A one liter preparation yielded approximately 2 mg of VP4.

Related Peptidases

According to the MEROPS protease database [28], all VP4 proteases belong to the evolutionary clan SJ. All members of this clan have an α/β protein fold and utilize a serine/lysine dyad in their catalytic mechanism. Lon-A Peptidase from *E. coli* also belongs to this clan (family S16) (Chapter 781). Due to a high level of sequence divergence, TV-1 VP4 is classified under protease family S69 instead of S50 to which IPNV, IBDV, DXV and BSNV VP4s belong. Although TV-1 was initially thought to be similar to IPNV, the sequence identity between their VP4s was only ~12%. TV-1 VP4 has less than 21% sequence identity with VP4s from BSNV, IBDV and DXV. Despite the low level of sequence identity, all birnavirus VP4 proteases solved to date share a similar overall tertiary structure. Other proteases that utilize the serine/lysine dyad mechanism include the bacterial proteases: signal peptidase I (family S26) [29] (Chapter 774), UmuD (Family S24) [30] (Chapter 773), and Repressor LexA (family S24) [31] (Chapter 772) from Clan SF and Signal peptide peptidase A (family S49) [32] (Chapter 798) and Tsp protease (family S41) [33] (Chapter 796) from Clan SK.

Further Reading

There are a number of reviews on proteases that utilize the serine/lysine catalytic dyad mechanism as well as other unusual serine proteases [34–36].

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