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Arterivirus Papain-like Proteinase 1α

Databanks

**MEROPS name:** porcine reproductive and respiratory syndrome arterivirus-type cysteine peptidase alpha  
**MEROPS classification:** clan CA, family C31, peptidase C31.001  
**Species distribution:** known only from lactate-dehydrogenase-elevating virus  
**Reference sequence from:** lactate-dehydrogenase-elevating virus (UniProt: Q83017)

Name and History

The family Arteriviridae currently includes the genetically distinct members equine arteritis virus (EAV; the family prototype), porcine reproductive and respiratory syndrome virus genotypes I and II (PRRSV-I and PRRSV-II), lactate dehydrogenase-elevating virus (LDV), and simian hemorrhagic fever virus (SHFV) [1]. EAV is the best-characterized arterivirus, although recent studies have increasingly been focused on PRRSV due to its economic importance. Arteriviruses are enveloped viruses with a polycistronic plus-strand RNA genome (12–15 kb; [2–6]). Their replicase proteins are expressed from open reading frames (ORFs) 1a and 1b that encode two large polyproteins: pp1a (187–260 kDa) and pp1ab (345–422 kDa), the latter resulting from a C-terminal extension of pp1a via ribosomal frameshifting. Both polyproteins are processed extensively by three or four ORF1a-encoded endopeptidases [7–15]. The arterivirus proteases and proteolytic pathways can be compared with those of the distantly related coronaviruses (see Chapters 494 and 546) and roniviruses, all of which are united in the order Nidovirales [16,17].

The papain-like proteinase 1α (PLP 1α), formerly also indicated as PCPα (for papain-like cysteine proteinase α), is the most N-terminally located member of an array of three (four in SHFV) cysteine protease domains that has been identified in the N-terminal 500 residues of arterivirus pp1a (see also Chapters 496 and 497). Its name derives from limited sequence similarity to the papain active site and the relative position of this proteinase in pp1α/pp1ab with respect to a downstream PLP (PLP 1β; Chapter 496). The PLP 1α domain is proteolytically active in the replicase polyproteins of PRRSV and LDV [11]. By cleaving the nsp1α→nsp1β junction, the proteinase mediates the autoproteolytic release of a 20–22 kDa N-terminal cleavage product named non-structural protein 1α (nsp1α), of which PLP 1α itself is part. In the arterivirus prototype equine arteritis virus (EAV), however, the
PLP 1α domain is not an active proteinase. As a result, the nsp1α and nsp1β equivalents of EAV reside in a single protein named nsp1, which is autocatalytically released from nsp2 by the activity of the PLP 1β autoproteinase domain (see Chapter 499) [7].

Activity and Specificity

The proteolytic activity of the PRRSV and LDV PLP 1α domains (and the inactivated state of the orthologous EAV domain) was initially deduced from comparative sequence analysis. This assignment was subsequently corroborated by analysis of processing products resulting from in vitro translation of RNA transcripts encoding the N-terminal region of the respective pp1a proteins [7,11]. Based on the apparent sizes of PRRSV nsp1α, PLP 1α had originally been proposed to cleave between 20 residues downstream of its catalytic His residue (His146). The crystal structure of recombinant PRRSV-II nsp1α, purified after its autoproteolytic release from a nsp1α/nsp1β precursor in E. coli, revealed that the protein spans 180 amino acid residues, suggesting that PLP 1α cleaves between Met180 and Ala181 in PRRSV pp1a/pp1ab [18]. N-terminal sequencing analysis of nsp1β that was immuno-precipitated was used to verify this suggestion. It from PRRSV-infected cells was used to verify this suggestion. IT confirmed Met180↓Ala181 as the site processed by PLP 1α to release nsp1α from the replicase polyproteins of PRRSV-II [19]. The nsp1α↓nsp1β cleavage site in PRRSV-I and LDV have not been identified to date.

In a rabbit reticulocyte lysate, PLP 1α was found to rapidly liberate nsp1α from the polypeptide generated in vitro, suggesting cleavage in cis [11]. Attempts to detect trans-cleavage activity in this system, as well as by assaying cleavage of short synthetic peptides by recombinant nsp1α in vitro [18], were unsuccessful. The occlusion of the PLP 1α active site by several nsp1α C-terminal residues, which was observed in the crystal structure of PRRSV nsp1α, may explain the lack of proteolytic activity subsequent to the self-processing event at the nsp1α↓nsp1β junction (see below).

Structural Chemistry

Comparative sequence analysis (Figure 495.1) suggests that the arterivirus PLP 1α domain spans approximately 120 amino acids and is fused with an N-terminal zinc finger (ZF) domain, with which it forms the ~180-residue nsp1α protein in LDV/PRRSV/SHFV or the N-terminal ~150 residues of the 260-residue nsp1 protein in EAV. The PRRSV and LDV PLP 1α domains contain a Cys and His residue pair with surrounding sequence characteristics typical for the Cys/His catalytic dyad found in viral papain-like proteinases [3,4,11,20]. The putative active-site Cys is followed, as usual, by a bulky, hydrophobic residue (Trp) and some local conservation was also detected. Replacement of Cys76 (the putative PLP 1α catalytic nucleophile in PRRSV and LDV) or His146 completely abolished cleavage of the nsp1α↓nsp1β site in a rabbit reticulocyte lysate. Combined with the sequence similarity to the active site of papain-like proteinases, these observations strongly suggested that these two residues form the PLP 1α catalytic dyad. This notion is strengthened by a recent report on the crystal structure of recombinant PRRSV-II nsp1α [18]. The PRRSV-II PLP 1α domain (Pro66 – Gln166 of nsp1α) was found to possess a typical peptidase clan CA papain-fold topology. It is composed of two opposing subdomains: one consisting of four left-handed α-helices and the other of three right-handed, antiparallel β-strands (Figure 495.2). Cys76 and His146 face each other at the interface of these two subdomains, and His146 is held in an orientation that would favor catalysis by hydrogen bonding to the side-chain oxygen atoms of Asn143 and Glu69. The C-terminal nsp1α residue — Met180, is positioned in the immediate vicinity of Cys76 and likely defines the S1 subsite of PLP 1α (Figure 495.2). PLP 1α loop regions connecting helices α3 and α4 and strands β4 and β5 shape the putative S2 subsite, occupied by Ala179, and multiple hydrophobic residues surrounding Phe176, the putative P5 residue. It is worthy of note that no less than eight of the C-terminal nsp1α residues are conformationally stabilized in the putative active site by a multitude of main-chain hydrogen bonds with PLP 1α residues. Together, the available structural and biochemical data strongly suggest that Cys76 and His146 form the PRRSV PLP 1α catalytic dyad, and that their counterparts Cys76 and His147 fulfill the same role in the LDV PLP 1α [11]; see alignment in Figure 495.1.

The crystal structure of PRRSV-II nsp1α confirmed the presence of a zinc finger (ZF) domain that had previously been identified in the N-terminal region of arterivirus nsp1 by bioinformatics [21]. The ZF, spanning residues Met1 to Glu65 of nsp1α, folds into two short parallel β-strands and a short α-helix around a zinc ion tetrahedrally coordinated by Cys8, Cys10, Cys25, and Cys28. The ZF topology places it in the structural super-family of β-β-α zinc fingers that are ubiquitous in cellular transcription factors [22].

Surprisingly, an additional zinc ion was found to be associated with the self-processed recombinant nsp1α following its crystallization. This ion is held in place by Cys76 and His146 together with Cys70 and Met180 (Figure 495.2). Given the nature of the peptide hydrolysis reaction by cysteine proteinases, zinc coordination by Cys76 and His146 would render them incapable of catalyzing this reaction, implying that this ion must be bound following nsp1α autoproteolysis. It is unclear what the significance of the second zinc ion could be for the
function(s) of nsp1 during viral replication, though it may help to stabilize the occlusion of the PLP 1 substrate-binding pocket by the nsp1 C-terminus.

Although the crystal structure of PRRSV-II nsp1 contained one monomer per asymmetric unit, in solution the protein was reported to form homodimers that displayed a remarkable resistance to high salt concentrations [18]. Molecular modeling of the nsp1 homodimer suggested that hydrophobic PLP 1 residues mediate the majority of contacts between the monomer subunits, though amino acids in the ZF domain are also likely to contribute to this interaction. The PLP 1 active sites face away from each other in the modeled dimer, which is compatible with a model of intramolecular autoproteolytic release of nsp1. The PRRSV nsp1 dimerization and its possible functional implications remain to be addressed in virus-infected cells. Interestingly, nsp1 of EAV has been demonstrated to form homo-oligomers during viral infection [23], and our recent unpublished data suggest that an autoproteolytically released recombinant nsp1 is also present largely in a dimeric form in solution. Dimerization may, therefore, be of significance for the post-proteolytic functions of these proteins in arterivirus replication.

Preparation

The nsp1-coding sequence of PRRSV-II was cloned into expression plasmid pET-28a and the construct was used to transform the BL21(DE3) strain of E. coli. The N-terminally His-tagged, self-processed nsp1 product was subsequently purified by metal affinity chromatography and used for structural studies [18].

Biological Aspects

The PLP 1-containing nsp1/nsp1 proteins of arteriviruses are accessory proteinases that assist the nsp4
main proteinase (see Chapter 692) in the proteolytic processing of the pp1a and pp1ab replicase polyproteins [16]. All available evidence points to only a single proteolytic event that is mediated by PLP 1α, i.e. the autoproteolytic release of nsp1α (see above). In combination with the multidomain organization of nsp1α and the loss of the PLP 1α proteolytic activity in EAV, this suggests that PLP 1α-containing arterivirus proteins have other, non-proteolytic functions during viral infection.

The loss of the proteolytic activity in the EAV PLP 1α lineage is, to our knowledge, almost unparalleled in virology (for the only other example, see Ziebuhr et al. [24]). It seems to be due to replacement of the catalytic Cys residue, and possibly other substitutions. However, despite this loss of proteolytic function and the low overall sequence similarity between EAV and PRRSV/LDV in this region, a number of PLP 1α residues have been conserved in the corresponding part of EAV nsp1 (Figure 495.1). This observation suggests the conservation of additional, non-proteolytic function(s) of this proteinase domain [11], and we recently obtained additional data supporting this view. In particular, EAV nsp1 was implicated in the selective regulation of subgenomic mRNA synthesis [21], which is a crucial, replication-dependent event in replicative cycles of arteriviruses and other nidoviruses [25–27]. Initially, EAV nsp1 was thought to exercise this function mainly through its ZF [21], but more recent data demonstrated the critical importance of charged residues from the PLP 1α (and PLP β) domains for subgenomic mRNA production [28]. EAV nsp1 also appears to fine-tune the abundance of each viral mRNA species by controlling the accumulation levels of its respective minus-strand template [28]. Finally, certain replacements in the ZF and PLP 1α domains of EAV nsp1 were found to interfere with virus production without affecting viral mRNA accumulation [28,29]. Thus, nsp1 seems to coordinate various key events in the EAV replicative cycle. PLP 1α residues have been implicated in all replicative functions of EAV nsp1, providing a possible explanation for the conservation of this proteolytically inactive domain. Interestingly, substitutions of catalytic PLP 1α residues that lead to a block in nsp1α autoproteolytic release render subgenomic mRNA accumulation undetectable in PRRSV-infected cells [30], suggesting that the key function of nsp1α/nsp1 in subgenomic mRNA synthesis is conserved among arteriviruses.

An intriguing aspect of nsp1/nsp1α biology is the fact that these proteins were found to partially localize to the cell nucleus during infection with EAV or PRRSV [19,31,32]. In the case of EAV nsp1, this appears to be due to active transport across the nuclear pore complex [31]. How these proteins are transported into the nucleus, in view of the absence of discernible nuclear localization signals in their primary structures, remains unclear. The relevance of the nuclear localization of nsp1/nsp1α during infection is currently unknown, but it may be connected to a recently described function of PRRSV nsp1α as an antagonist of Type I interferon (IFN), the synthesis and secretion of which are key events of cellular innate immune responses. Overexpression of nsp1α (and nsp1β, see Chapter 496) strongly inhibited the expression of a reporter gene driven by an IFNα promoter in the absence of other viral proteins [19,32–34]. Subsequent reports provided evidence for a function of PRRSV nsp1α as a negative modulator of NF-κB activation, an important regulatory step leading to expression of various immunomodulatory factors, including IFN [32,35]. The protein domains responsible for the suppression of innate immune responses by PRRSV nsp1α have not yet been delineated, nor has the relevance of this proposed function for virus infection been examined. Interestingly, nsp1α does not
seem capable of inhibiting cellular responses to IFN, unlike nsp1α, which suppresses IFN synthesis, as well as subsequent IFN-mediated signaling events ([19], see Chapter 496).

**Distinguishing Features**

PLP 1α is a small papain-like cysteine proteinase domain, which is linked to an N-terminal zinc finger domain (Figure 499.2). It cleaves the arterivirus pp1a and pp1ab replicase polyproteins in cis ~35 residues downstream of its active-site His residue, and thus releases the N-terminal nsp1α subunit of the PRRSV and LDV replicase. The proteolytic activity of PLP 1α appears to have been lost during EAV evolution.

A polyclonal rabbit antiserum against EAV nsp1 was raised by immunization with a peptide representing the first 23 residues of pp1a [8]. This serum is available from the authors for research purposes on request. The production of a mouse monoclonal antibody (12A4) recognizing EAV nsp1α antisera directed against nsp1α of its active-site His residue, and thus releases the N-terminal nsp1α subunit of the PRRSV and LDV replicase. The proteolytic activity of PLP 1α appears to have been lost during EAV evolution.

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**Related Peptidases**

The PLP 1α lineage may have emerged by duplication in an ancestor of arteriviruses. In evolutionary terms it is far separated from other papain-like peptidases.

**Further Reading**

The papers of den Boon et al. [11], Ziebuhr et al. [16], Tijms et al. [21], Sun et al. [18], Chen et al. [19], and Nedalkova et al. [28] are recommended.

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