Peptides resulting from the pVP2 C-terminal processing are present in infectious pancreatic necrosis virus particles

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The complete proteolytic cascades involved in processing the IBDV and BSNV polyproteins and generating VP2, the structural peptides and VP3 have been characterized. The polyproteins are cotranslationally cleaved to generate pVP2 (the precursor of VP2), VP4 and VP3. Cleavage sites have been identified at the pVP2–VP4 and VP4–VP3 junctions (Sanchez & Rodriguez, 1999; Lejal et al., 2000; Da Costa et al., 2003). For both viruses, processing of pVP2 generates VP2 and four small peptides derived from the C terminus of pVP2 (Da Costa et al., 2002, 2003). These peptides are associated with the virus particles. For IPNV, only the VP4 target sites generating pVP2, VP4 and VP3 have been defined, being between aa 508–509 and 734–735 of the polyprotein (Petit et al., 2000). In this study we characterized the final processing of the IPNV polyprotein. By using mass spectrometry and N-terminal sequencing, we identified peptides derived from the C-terminal domain of pVP2 that were present in the virus particles.

Fig. 1(a) shows a multi-alignment of the C-terminal domains of the birnavirus pVP2s. The sequence comparison is anchored to the multiple cleavage sites identified in the C terminus of pVP2 of BSNV and IBDV and to the cleavage site mapped at the pVP2–VP4 junction of IPNV and DXV (Chung et al., 1996; Sanchez & Rodriguez, 1999; Petit et al., 2000; Da Costa et al., 2002, 2003). We predicted from this alignment that pVP2 processing of IPNV might result in the generation of mature VP2 (aa 1–442 of the polyprotein) and three peptides (aa 443–486, 487–495 and 496–508 of the polyprotein). The [M+H]+ theoretical monoisotopic masses of the three peptides were expected to be 4796.59, 949.43 and 1451.73 Da, respectively. As most IBDV and
BSNV pVP2-derived peptides have been identified on virus particles, we postulated that their IPNV homologues should be identifiable in purified IPNV virions.

IPNV used in this study (strain 31-75, serotype Sp; Dorson et al., 1978) was propagated in the rainbow trout gonad-2 cell line. The genome of this strain has been cloned and sequenced (database accession numbers AJ622822 and AJ622823). To identify the predicted peptides by mass spectrometry, the virus particles were purified by density centrifugation as shown in Fig. 1(b) (left panel). Two gradients are shown, resulting from two different purification procedures (see below). Four main bands were observed in the CsCl gradients. Electron microscopy revealed that the three lower bands (1, 1', and 1") contained typical birnavirus particles, and the upper band (2) contained particles with a diameter of about 25 nm (Fig. 1b, middle panel). SDS-PAGE showed that the virus particles contain the expected VP1 (90 kDa), VP2 (54 kDa) and VP3 (31 kDa) structural proteins, and a very small amount of pVP2. The difference in density between the virus particles present in bands 1, 1', and 1" could be explained by differences in the amount of nucleic acid present in particles. An analogous band profile has been described for another birnavirus, IBDV (Muller & Becht, 1982; Da Costa et al., 2002).

Mass spectrometry analysis was carried out on the virus particles present in band 1 using a Voyager-DE STR time-of-flight mass spectrometer (Applied Biosystems). One
main peptide with a monoisotopic \([M+H]^+\) mass of 1451-72 was identified (Fig. 2a, top panel). This \([M+H]^+\) mass fitted well with the theoretical mass of the predicted 13 aa long peptide extending from Ala-496 to Ala-508, which is 1451-73 Da. As cleavage at the pVP2–VP4 junction occurs between aa 508 and 509, this peptide is indeed the C terminus of pVP2. An additional peptide with an \([M+H]^+\) mass of 4796-14 was detected (Fig. 2a, lower panel). No other peptide with the same range of mass (3500–5500 Da) was detected. This \([M+H]^+\) mass fitted well with the mass of a peptide extending from Trp-443 to Ala-486, which is 4796-59 Da and represents the N-terminal part of this pVP2 domain. The presence of these two peptides from residues [443–486] and [496–508] in the virus particles prompted us to locate the putative 9 aa peptide derived from residues 487–495. To identify this peptide, which had a theoretical \([M+H]^+\) monoisotopic mass of 949-43 Da, we analysed the magnified signal in this mass range. As shown in Fig. 2(a) (top panel), a peptide with an \([M+H]^+\) monoisotopic mass of 949-61 was detectable. Fourteen additional peptides were also identified by mass spectrometry in the mass range 590–2300 Da (Fig. 2a, top panel). Thirteen of these appeared to be cleaved products derived from peptide [443–486] (Fig. 2b, c). All possessed the same N terminus as peptide [443–486]. No complementary peptide harbouring the corresponding C-terminal domains of peptide [443–486] was identified. These observations suggest that a fraction of peptide [443–486] was cleaved by a carboxypeptidase with low specificity. A cellular carboxypeptidase, or possibly VP4, might be associated with these cleavage events. Finally, a peptide, with an \([M+H]^+\) mass of 1380-70, deriving from the amino- or carboxy-cleavage of peptide [496–508], was also detected.

To confirm the existence of these peptides in the virus particles, we carried out N-terminal sequencing on the
purified virus, as described for IBDV (Da Costa et al., 2002). Because the main capsid proteins, VP2 and VP3, have been reported to possess a blocked amino terminus (Dobos, 1995), only the N termini of the peptides should be detected. The results are presented in Fig. 3. The N-terminal amino acids of peptide [443–486] were identified through 15 Edman degradation cycles. In particular, residue Trp at positions 1 and 3, and residues Asp and Ile at positions 5 and 6, respectively, provided a signature for this peptide. In addition, an Ala at position 1 and a Gly at positions 2 and 3 were revealed, compatible with the predicted N-terminal sequences of both peptides [496–508] and [487–495]. The presence of these short peptides was validated by the identification of residues (His, Lys), (Ser, Asp), (Met, Val) and (Ala, Leu) at positions 6 to 9, respectively. Thus N-terminal sequencing results were consistent with the mass spectrometry analysis. In summary, three peptides corresponding to amino acid residues [443–486], [487–495] and [496–508] were detected in virus particles. A large number of peptides deriving from carboxy-terminal processing of peptide [443–486] were also detected.

Two procedures were used to purify IPNV particles (Fig. 1b, left panel). Before density centrifugation in CsCl, the virions were concentrated by either ultracentrifugation or low-speed centrifugation after overnight incubation in 10% polyethylene glycol 6000 (PEG). When the virus was ultracentrifuged, band 2 was denser than when the material had been concentrated with PEG. In contrast, the virus bands contained more material after PEG treatment. Electron microscopy revealed isometric particles in band 2 (Fig. 1b, middle). SDS-PAGE (Fig. 1b, right) and mass spectrometry analysis (see below) showed that these particles contained only VP2. These results suggest that the IPNV virus particles were partially altered by ultracentrifugation, and the dissociated VP2 self-assembled into structures that did not contain VP3.

The existence of peptides derived from pVP2 suggests that aa [443–508] represent the domain that is processed during pVP2 maturation. To determine whether the pVP2-processed domain involved residues upstream of residue 442, we engineered a baculovirus driving expression of the IPNV polyprotein with a stop codon at position 443, and compared the electrophoretic mobility of the mature VP2 (present on virus particles) with this truncated form. To this end, plasmid SK-AIPNA (Petit et al., 2000) was used as a template to amplify the VP2 reading frame (the first 442 amino acids (single-letter code) and amount (in differential picomoles, the amount in picomoles of an amino acid at one position after background subtraction of its amount detected at the previous position) revealed at each Edman degradation position after background subtraction of its amount detected at the previous position) revealed at each Edman degradation cycle (1–15). The sequences of the three peptides are aligned on the residues identified.

![Fig. 3. N-terminal sequencing of the purified virus. (a) Sequence of the IPNV pVP2 C-terminal domain (aa 443–508 of the polyprotein). Arrows indicate cleavage sites. (b) Nature of amino acids (single-letter code) and amount (in differential picomoles, the amount in picomoles of an amino acid at one position after background subtraction of its amount detected at the previous position) revealed at each Edman degradation cycle (1–15). The sequences of the three peptides are aligned on the residues identified.](image-url)
(Petit et al., 2000). These two sites, and the primary cleavage site at the pVP2–VP4 junction, were defined by the motif [S/T]X[A]↓A. This consensus sequence shared some similarity with the sequence SKA↓W surrounding the maturation cleavage site at position 442–443, suggesting that VP4 could be involved in the cleavage generating the mature VP2.

When the virus particles were altered by ultracentrifugation, the released VP2 was able to self-assemble into particles with a diameter of about 25 nm. These observations suggest that the VP2 of IPNV has assembly properties similar to the VP2/pVP2 of IBDV (Martinez-Torrecuadrada et al., 2000; Caston et al., 2001; Chevalier et al., 2002).

The role of the birnavirus structural peptides remains to be elucidated. We favour the hypothesis that they may be involved in virus entry into the target cells, but it cannot be ruled out that they play a role in capsid assembly or genome encapsidation.

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References


