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Peptide Map Comparison of the Proteins of Infectious Bursal Disease Virus

PETER DOBOS

Department of Microbiology, College of Biological Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada

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The genome of infectious bursal disease virus consists of two segments of double-stranded RNA of 2.5×10^6 and 2.2×10^6 molecular weight. Polyacrylamide gel electrophoresis of purified virus resolved four structural polypeptides: VP-1 (90,000), VP-2 (41,000), VP-3 (35,000), and VP-4 (28,000). Peptide map comparisons of radioiodinated virion proteins indicated no precursor-product relationship between them. The possible relationship between the size of the virus genome and the number and sizes of different viral proteins is discussed.

Infectious bursal disease virus (IBDV) is the causative agent of infectious bursal disease (Gumboro disease) of chickens (8). It is a naked icosahedral virus with an electron microscopic diameter of 60 nm (8). As has been demonstrated recently, the virus genome consists of two segments of high-molecular-weight double-stranded RNA (2.2×10^6 and 2.5×10^6) (7). With respect to size, morphology, and the bisegmented nature of the virus genome, IBDV is similar to a number of other unclassified viruses, such as infectious pancreatic necrosis virus of fish (3, 12), tellina virus of bivalve molluscs (12), and drosophila X virus of *Drosophila melanogaster* (11).

So far there has been only one report, by Nick et al., which described the proteins of IBDV (8). They have analyzed the polypeptides of purified virus in 7.5% polyacrylamide gels using the urea-phosphate-sodium dodecyl sulfate (SDS) buffer system of Smith et al. (10) and were able to resolve four to six proteins: two minor polypeptides, VP-1 (110,000) and VP-2 (50,000), and two major ones, VP-3 (35,000) and VP-4 (25,000). Sometimes VP-2 appeared as a double band, and some preparations produced a minor band (VP-0) in a position corresponding to a molecular weight of approximately 180,000. The combined molecular weight of the virion proteins (440,000) exceeds the coding capacity of the virus genome (a maximum of 230,000-molecular-weight protein), indicating that some of these polypeptides may not be primary gene products. The discrepancy between the number and sizes of virion proteins and the size of the bisegmented double-stranded virus genome raised the following question. How can a large-size class double-stranded RNA genome mediate the synthesis of three different size classes of proteins (large, VP-0 and VP-1; medium, VP-2 and VP-3; and small, VP-4)? The general mechanism observed with reovirus, where each genome segment codes for a polypeptide via a monocistronic mRNA, cannot operate here since IBDV contains no medium- and small-size class genomic double-stranded RNAs.

One possible explanation is that VP-2, VP-3, and VP-4 are post-translational cleavage products of VP-1. This theory is especially attractive because the combined molecular weights of VP-2, VP-3, and VP-4 happen to be equal to that of VP-1. (The occasional VP-0 may represent dimerized VP-1.) The minor protein VP-1 may represent residual, uncleaved precursor encapsidated into the virion, similar to polypeptide ϵ in encephalomyocarditis virus where ϵ is cleaved to produce virion proteins β and δ (9). If such a mechanism were to operate during the morphogenesis of IBDV, then the tryptic peptide map of VP-1 would overlap with those of VP-2, VP-3, and VP-4, indicating that one of the genome segment codes for VP-1 and the other probably codes for nonstructural virus-specific proteins. If, however, the peptide maps of the four virion polypeptides were different from one another, indicating no precursor-product relationships, then these structural proteins would account for the total coding capacity of the virus genome. To determine which is the case, tryptic digests of isotopically labeled proteins from purified virus were compared by two-dimensional peptide mapping.

Plaque-purified IBDV strain Cu-1 (8) was propagated in chicken embryo cell monolayers and purified by Freon extraction, pelleting, and one cycle each of sucrose gradient and CsCl

isopycnic gradient centrifugation as described by Nick et al. (8). To prepare ultrapure virus, some preparations were purified by two cycles of sucrose gradient and CsCl gradient centrifugation. The virion proteins were analyzed with 10% polyacrylamide slab gels in the discontinuous SDS gel system described by Laemmli (6). For peptide-map comparison, the Coomassie brilliant blue-stained polypeptides were sliced out of the gel, radioiodinated in vitro according to the method of Elder et al. (5), and subjected to tryptic digestion and two-dimensional peptide mapping followed by autoradiography as described previously (4).

The virus genome was analyzed in 5% polyacrylamide slab gels as described before (1). After the run, the gel was stained with ethidium bromide solution and photographed under UV light.

The protein gel pattern of purified virus is shown in Fig. $1B_1$. Four polypeptides were resolved, VP-1 (90,000), VP-2 (41,000), VP-3 (35,000), and VP-4 (28,000). An additional polypeptide in the form of a doublet (VP-X) was also observed, but the amount of this protein varied in different preparations, suggesting that it may have been a "contaminant" protein adhering to the virus. To test this possibility, IBDV was purified by two cycles of sucrose gradient and CsCl gradient centrifugation, and when this preparation was analyzed in polyacrylamide gels, the VP-X protein was absent (Fig. $1B_2$).

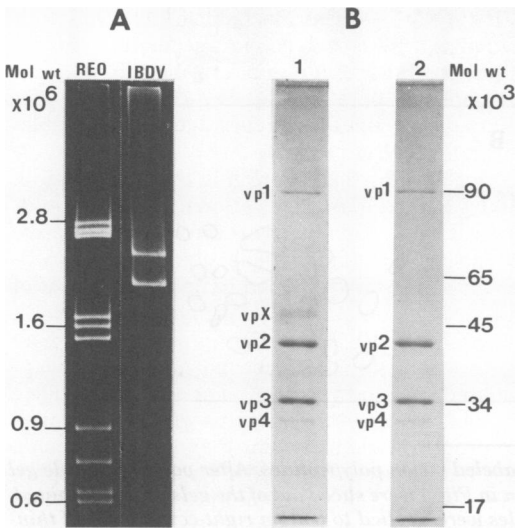

FIG. 1. *Virion RNA and proteins of IBDV. (A) Polyacrylamide gel electrophoresis of IBDV RNA, together with reovirus RNA in 5% polyacrylamide gel, followed by staining with ethidium bromide. (B) Polyacrylamide gel electrophoresis of purified IBDV in 10% gels using the discontinuous SDS-gel system described by Laemmli (6). The gels were stained with Coomassie brilliant blue. The position and the molecular weight × 10^3 of the marker proteins are shown on the right and those of the virion proteins are shown on the left. The marker proteins used were phosphorylase A (90,000), bovine serum albumin (65,000), ovalbumin (45,000), aspartate transcarbamylase (34,000), and tobacco mosaic virus protein (17,000). (B_1) IBDV purified by one cycle of sucrose gradient and CsCl gradient centrifugation; (B_2) IBDV purified by two cycles of sucrose gradient and CsCl gradient centrifugation.*

The data presented in Table 1 summarize the identities of virion proteins shown in Fig. $1B_1$ as compared with those reported by Nick et al. (8). I have detected an additional minor protein, VP-4 (28,000), which has not been reported previously, which may represent the minor undesignated protein shown by Nick et al. on the absorbance tracing of a stained gel which migrated faster than their VP-4 (8). I detected no minor polypeptide of 180,000 molecular weight in any of the preparations examined.

The data in Fig. 2 show that the two-dimensional peptide maps of VP-2, VP-3, and VP-4 are different from one another. Their relationship to that of VP-1 is difficult to assess by visual comparison of the four autoradiograms. Therefore, for better comparison, the spots of VP-1 were traced onto paper (panel A and B, solid lines) and superimposed on tracings of spots of VP-2 and VP-3, plus VP-4 (dashed lines). This kind of comparison is valid since digests to be compared were subjected to electrophoresis together in an apparatus that accommodated two silica gel plates simultaneously. The limited amount of VP-1 polypeptide available (or its low specific activity or both) did not allow the analysis of mixed digests of VP-1 and VP-2 or VP-1 and

TABLE 1. *Comparison of IBDV polypeptides analyzed in two different gel systems*[a]

SDS-phosphate gel[b]		SDS-Tris-glycine gel[c]	
Polypeptide	Mol wt	Polypeptide	Mol wt
VP-1	110,000	VP-1	90,000
VP-2[d]	50,000	VP-X[d]	47,000
VP-3[e]	35,000	VP-2[e]	41,000
VP-4[e]	25,000	VP-3[e]	35,000
		VP-4	28,000

[a] The polypeptide pattern of IBDV, analyzed according to the Laemmli discontinuous gel system (6), is compared to that of Nick et al. (8) who used the SDS-phosphate gel system described by Smith et al. (10).

[b] As determined by Nick et al. (8).

[c] As shown in Fig. $1B_1$.

[d] Minor polypeptide appearing as a double band.

[e] Major polypeptide.

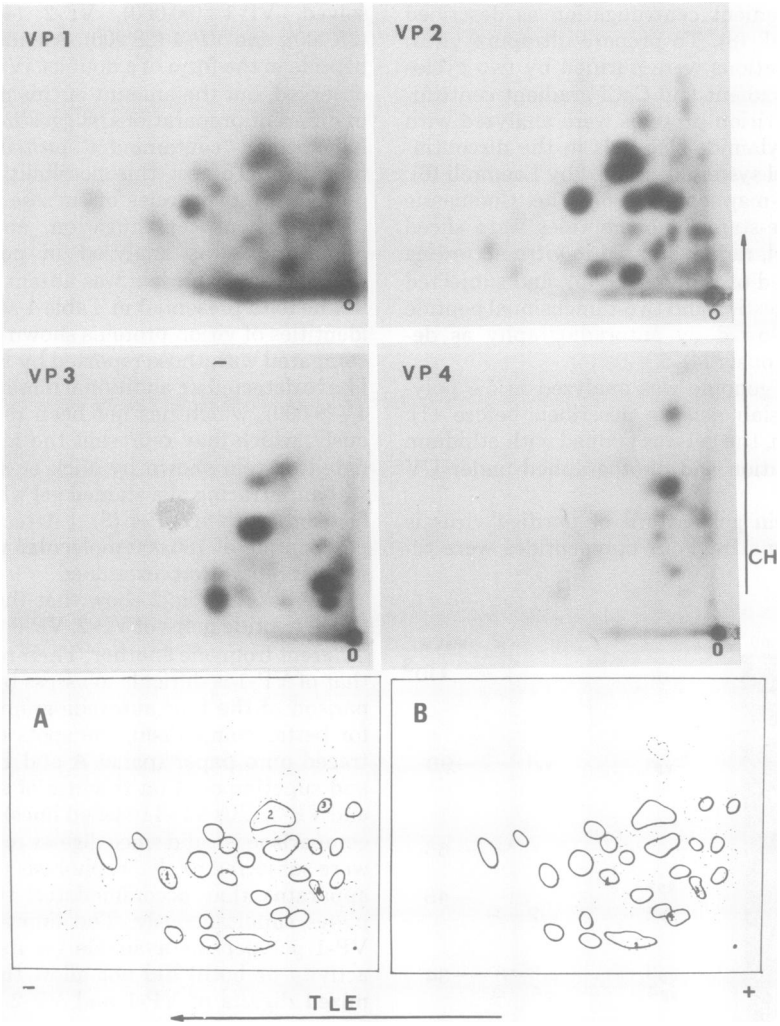

FIG. 2. *Two-dimensional tryptic peptide maps of ^{125}I-labeled virion polypeptides. After polyacrylamide gel electrophoresis, the stained bands of virion proteins shown in Fig. 1 were sliced out of the gels, radioiodinated, and digested with trypsin as described in the text. Samples were applied to bottom right corners (O) of thin-layer silica gel plates. Electrophoresis was at pH 3.5 with the anode to the right. Phenol red was spotted onto the left-hand corner of the plate to serve as visual marker, and the digests were subjected to electrophoresis at 300 V until the phenol red spot moved 8 cm toward the anode. For direct comparison, the two samples to be compared were subjected to electrophoresis in parallel by using an electrophoretic apparatus that accommodated two thin-layer silica gel plates (20 by 20 cm) simultaneously. The dried plates were exposed to X-ray film in the dark for 7 days (VP-2, VP-3, VP-4) and 21 days (VP-1). Panel A shows the tracing of peptide spots of VP1 (solid line) and that of VP-2 (dashed line). Panel B shows the tracings of peptide spots of VP-1 (solid line) and those of VP-3 and VP-4 (dashed line). The numbers in both panels indicate overlapping peptides. Some of the spots shown on the tracings were weak and therefore may be difficult to see in the autoradiograms; however, they were readily detectable on the original X-ray films from which panels A and B were traced. TLE, thin-layer electrophoresis; CH, chromatography.*

VP-3 plus VP-4 on the same plate without causing streaking due to overloading. Of the 43 peptides of VP-2, only 4 overlapped with the 24 peptides of VP-1 (see spots marked from 1 to 4 in panel A). Similarly, only four peptides of VP-1 overlapped with those of VP-3 and VP-4, which contained 17 and 22 peptides, respectively (a total of 39). These results argue against VP-1 being a precursor of VP-2, VP-3, and VP-4.

Furthermore, since the amount of iodination depends on the number of histidine, phenylalanine, and tyrosine residues (5), VP-1 should con-

tain as many of these amino acids as VP-2, VP-3, and VP-4 together if it were the precursor protein of these smaller polypeptides; yet digests of VP-1 contained 24 iodinated peptides, whereas the total number of peptides found in the digests of VP-2, VP-3, and VP-4 was 82 (see tracings in panels A and B, Fig. 2). These results, again, make VP-1 an unlikely candidate for a precursor of VP-2, VP-3, and VP-4.

On the other hand, it could be argued that weak spots in the digests of VP-2, VP-3, and VP-4 are below detectable levels in the digest of the putative precursor VP-1 because of the small amount (or low specific activity) of VP-1 that was available from virion proteins separated by polyacrylamide gel electrophoresis. It is also possible that if cleavage of VP-1 took place to generate the smaller proteins, it was accompanied by post-translational modifications of the polypeptides such as sulfurylation, glycosylation, phosphorylation, acetylation, and methylation, which would have changed the electrophoretic or chromatographic behavior or both, of a number of peptides of VP-2, VP-3, and VP-4 and would account for the low number of overlapping spots of the digests of these proteins and their putative precursor VP-1. Although these possibilities cannot be discounted, the available evidence favors the theory that VP-1 is not a precursor of VP-2, VP-3, and VP-4.

When VP-X was analyzed by peptide mapping, it was found to be qualitatively identical to VP-2 (Fig. 3). This seemed to indicate that VP-2 was a cleavage product of VP-X. The justification for this supposition comes from studies of intracellular virus-specific protein synthesis in infectious pancreatic necrosis virus-infected cells (2). Here we also found occasionally a minor protein component in purified virus that migrated in gels somewhat slower than the major polypeptide VP-2 (called polypeptide β by a previous nomenclature; 2). Subsequent studies, involving pulse-chase experiments and peptide mappings of all intracellular virus-specific polypeptides, revealed that VP-2 of infectious pancreatic necrosis virus was produced by post-translational "trimming" of a slightly larger polypeptide in a two-step cleavage process during virus maturation (2, 4). This precursor protein was sometimes present in purified virus preparations, which was not surprising considering that most of the progeny virus remained cell associated and had to be extracted by Freon from infected cultures. The precursor protein could be eliminated only by repeated cycles of sucrose gradient and CsCl gradient centrifugation. Although this analogy does not prove that a similar mechanism operates in IBDV-infected

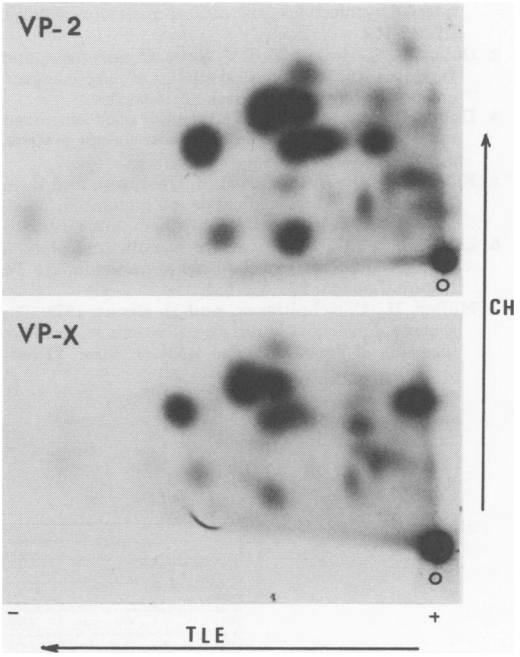

FIG. 3. *Tryptic peptide maps of digests of ^{125}I-labeled VP-X and VP-2 polypeptides. Conditions for tryptic digestion, electrophoresis, and chromatography were as described in Fig. 2, except that the two samples to be compared were subjected to electrophoresis in parallel on the same thin-layer plate which was cut in half for subsequent chromatography.*

cells, the near-identical peptide maps of VP-X and VP-2, and the fact that IBDV and infectious pancreatic necrosis virus share many biochemical and biophysical characteristics add weight to this argument.

Considering the molecular weights of the two genome segments and the molecular weights of virion proteins, it may be postulated that one of the RNA segments code for VP-1 and the other codes for VP-2, VP-3, and VP-4. The latter three proteins could arise by proteolytic cleavage of large nonstructural precursor polypeptide or they could be produced by translation of monocistronic, subgenomic-size mRNA's. Whatever the case, IBDV probably has a unique mechanism to synthesize three size classes of polypeptides by using mRNA transcripts from two high-molecular-weight double-stranded RNA genome segments.

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