Protein Coding Assignment for the Genome of Epizootic Haemorrhagic Disease Virus

By JAMES O. MECHAM and VICTORIA C. DEAN
Agricultural Research Service, Arthropod-borne Animal Diseases Research Laboratory, P.O. Box 3965, University Station, Laramie, Wyoming 82071-3965, U.S.A.

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SUMMARY

Viral genomic RNA was purified from BHK-21 cells infected with epizootic haemorrhagic disease virus and the 10 dsRNA genome segments were isolated by polyacrylamide gel electrophoresis. These genome segments were translated in vitro using the rabbit reticulocyte lysate system and the synthesized proteins were detected by immune precipitation and gel electrophoresis. This allowed the assignment of protein coding to the genome segments and the identification of two additional virus-specified proteins not readily detectable in lysates of virus-infected cells.

INTRODUCTION

Epizootic haemorrhagic disease virus (EHDV) is a member of the Orbivirus genus of the family Reoviridae (Borden et al., 1971; Matthews, 1982). EHDV causes acute disease in both domestic and wild ruminants (Shope et al., 1960; Metcalf & Luedke, 1980; Couvillon et al., 1981). The genome of EHDV is composed of 10 segments of dsRNA (Huismans et al., 1979; Kontor & Welch, 1976; Knudson et al., 1982; Kusari & Roy, 1986). Between eight and 12 EHDV structural and/or non-structural proteins have been tentatively identified by polyacrylamide gel electrophoresis (PAGE) of purified virions and infected cell extracts (Huismans et al., 1979; Kusari & Roy, 1986). The precise identification of viral proteins has been prevented by the presence of cellular proteins and relatively small amounts of certain viral proteins in the purified virions or infected cell extracts. In addition, some proteins identified by PAGE may not be unique viral proteins but may be cleavage products of other proteins.

In vitro translation of dsRNA to determine the protein coding assignment of RNA segments was first accomplished with reovirus (McCrae & Joklik, 1978). In vitro translation of the dsRNA of bluetongue virus (BTV), which is an orbivirus containing 10 dsRNA genome segments (Verwoerd, 1969; Verwoerd et al., 1970), has permitted the protein coding assignment of the individual genome segments (Grubman et al., 1983; Mertens et al., 1984). This information has been of value in determining which genome segments code for viral proteins possessing important biological epitopes such as group- or serotype-specific antigenic determinants.

It is not known which genome segments code for which EHDV proteins. To discover which segments code for the proteins the RNA of EHDV was isolated and translated in vitro using the rabbit reticulocyte lysate system. This permitted the identification of 10 virus-specified proteins and determination of the coding assignment of the individual genome segments.

METHODS

Virus and cell culture. The Alberta strain (1962) of EHDV was plaque-purified and grown in BHK-21 cell culture. BHK-21 cells were obtained from the American Type Culture Collection and were grown in Eagle's minimal essential medium containing penicillin (100 units/ml), streptomycin (100 μg/ml) and 10% foetal bovine serum.
Translation of unfractionated dsRNA

EHDV dsRNA was extracted from infected BHK-21 cells, denatured with methylmercuric hydroxide and translated using the rabbit reticulocyte lysate system (Fig. 1). An RNA concentration of approximately 40 to 50 μg/ml was found to be optimal. In vitro translation products were observed with electrophoretic mobilities identical to the viral structural proteins designated VP1, VP2, VP3, VP4, VP5 and VP7 and to a non-structural viral protein designated NS1. A protein synthesized in vitro with a mobility equal to that of VP6 was not observed. Proteins were also produced by in vitro translation that were not readily observed in the lysates.
from infected cells. It was necessary to denature the viral dsRNA with methylmercuric hydroxide for 10 min at 37 °C and to carry out in vitro translation for 3 h to obtain adequate production of VP1, VP2 and VP3. Even under these conditions, VP1 was produced in very small amounts.

**Translation of individual species of dsRNA**

Total genomic dsRNA was fractionated by preparative PAGE as described in the Methods. All of the genome segments except 7 and 8 could be resolved. Numerous attempts to separate these two segments using various gel systems were unsuccessful. The purity of the isolated segments was determined by PAGE and silver staining (Fig. 2). There was relatively little cross-contamination of the individual isolated species of dsRNA. The individual RNA species were translated in vitro as described.

Fig. 3 shows the results of translations of all 10 isolated dsRNA segments. Part (a) shows the results of translation of segment 1. Translation of this segment produced a major high $M_r$ product which comigrated with VP1 from infected cell extracts. Fig. 3(b) shows the results of translation of the remaining genome segments. Genome segments 2 and 3 coded for major proteins comigrating with VP2 and VP3, respectively. Some cross-contamination of segments 2 and 3 appeared to have occurred during isolation of these segments. Translation of segments 1, 2 and 3 produced a number of minor proteins migrating below the major protein species. Segments 4 and 5 coded for proteins comigrating with VP4 and VP5, respectively. Segment 6 coded for a protein comigrating with NS1. Genome segments 7 and 8 were translated together and coded for a protein comigrating with VP7 and for another protein migrating between NS1 and VP6.
Segment 9 coded for two proteins migrating between NS1 and VP6. The upper protein of this pair comigrated with one of the proteins coded for by segments 7/8 and was identical to it (as determined by peptide mapping), suggesting that synthesis of this protein was a result of cross-contamination of segment 9 with one of these other two segments. Attempts to enhance \textit{in vitro} translation of segment 9 and decrease cross-contamination by varying the experimental conditions were unsuccessful. Finally, translation of genome segment 10 produced a protein that migrated approximately halfway between VP7 and the dye front. This protein was produced in much lower amounts in infected cells than in the reaction \textit{in vitro}. None of the \textit{in vitro} translation products comigrated with VP6.

\textbf{Peptide mapping of translation products}

Peptide mapping of the proteins produced by \textit{in vitro} translation was performed to confirm the RNA coding assignments based upon their relative migration by PAGE as described above. The PAGE profiles of peptides generated by staphylococcal V8 protease cleavage of proteins synthesized \textit{in vitro} were compared to the viral proteins with which they were postulated to be identical. The translated proteins postulated to correspond to VP2, VP3, VP4, VP5 and VP7 gave identical peptide map profiles to viral proteins from infected cells (Fig. 4). It proved impossible to obtain adequate amounts of the protein translated \textit{in vitro} that was postulated to correspond to VP1 to permit peptide mapping. Peptide mapping also indicated that the NS1 synthesized \textit{in vitro} was identical to the NS1 synthesized \textit{in vivo} (Fig. 5). NS1a, which is produced in infected cells but not during \textit{in vitro} translation, is related to NS1 (Fig. 5c). Peptide mapping of the two \textit{in vitro} translation products migrating between NS1 and VP7 suggested that the product of segment 9 was related to VP6 synthesized \textit{in vivo} (Fig. 6). It was not possible to compare the \textit{in vitro} product of segment 10 with its \textit{in vivo} counterpart by peptide mapping because only small amounts of this protein were synthesized in infected cells.
Fig. 3. *In vitro* translation of the individual dsRNA segments of EHDV. Individual segments of viral dsRNA were isolated and translated *in vitro*. (a) Lane 1, viral proteins from infected cells; lanes 2, 3, 4, *in vitro* translation of total EHDV dsRNA, segment 1 and no added exogenous RNA, respectively. (b) Lane 1, viral proteins from infected cells; lanes 2 and 11, *in vitro* translation of total EHDV dsRNA; lanes 3 to 10, *in vitro* translation of segments 2, 3, 4, 5, 6, 7/8, 9 and 10, respectively; lanes 12 and 13, *in vitro* translation of myoglobin mRNA and no exogenous RNA, respectively. Viral proteins from infected cells or from *in vitro* translation reactions were labelled with [35S]methionine, immune-precipitated with rabbit antiserum to EHDV, and analysed by PAGE and autoradiography.

Fig. 4. Peptide mapping of EHDV proteins synthesized *in vitro* (1) and *in vivo* (2). Plugs containing the specified proteins were cut from preparative gels and wedged into the wells of a 15% polyacrylamide gel. Protease digestion was carried out *in situ* during electrophoresis using 5 μg/well of V8 protease.
Fig. 5. Peptide mapping of NS1 synthesized \textit{in vitro} (a) and \textit{in vivo} (b) and NS1a synthesized \textit{in vivo} (c). The isolated proteins were digested with 5 μg of V8 protease and the peptide fragments were separated by PAGE. The autoradiographs were scanned from top to bottom with an LKB laser densitometer. The top of each autoradiograph is at the left of each figure.

Fig. 6. Peptide mapping of the \textit{in vitro} translation products of EHDV genome segments 7/8 (a), segment 9 (b) and VP6 from EHDV-infected cells (c). Protease digestion and analysis of the viral proteins were carried out as described in Fig. 4.

DISCUSSION

The entire genome of EHDV codes for 10 viral proteins. A unique protein is encoded by each segment (Fig. 7). Proteins corresponding to VP1, VP2, VP3, VP4, VP5, NS1 and VP7, as determined by PAGE, immune precipitation, and peptide mapping were produced by \textit{in vitro} translation of viral dsRNA. \textit{In vitro} translation of genome segments 7/8 and 10 produced two proteins not apparent in infected cells. These proteins were designated non-structural because they were not observed in purified virus. The proteins from segments 2 and 3 (VP2 and VP3) were produced in relatively smaller quantities \textit{in vitro} than \textit{in vivo}. Translation of segments 1, 2
and 3 produced numerous minor polypeptides, which may represent premature translation products of these larger RNAs, migrating below the major protein products. Similar observations have been made during in vitro translation of the larger genome segments of BTV type 1 (Mertens et al., 1984). The protein products of genome segments 4, 5, 6 and 7 (VP4, VP5, NS1 and VP7) were quantitatively similar both in vitro and in vivo.

On the basis of peptide mapping the viral protein designated NS1a appears to be related to NS1. The lack of NS1a production during in vitro translation may indicate that a cellular protease is responsible for its production from NS1 during virus replication in infected cells. Similarly, the protein produced by in vitro translation of genome segment 9, which is similar by peptide mapping to VP6, may be processed intracellularly to form VP6.

Mertens et al. (1984) have suggested that some type of regulatory mechanism may reduce the relative level of expression of BTV genome segment 10 in vivo compared to its expression in vitro. We have observed a similar discrepancy with regard to segment 10 of EHDV. The observation that segment 9 of EHDV was translated less well in vitro than other small RNA species, even though present in similar amounts, adds further support to the idea that some regulatory mechanism may control the levels of translation of certain genome segments.

The genome coding assignment of EHDV closely resembles that of BTV type 1 (Mertens et al., 1984) and type 17 (Grubman et al., 1983). In general, there was a good correlation between the size of genome segments and the proteins for which they coded. The exceptions to this rule were seen with segments 7/8 and 9. Segments 7/8 coded for at least one protein (VP7) which was smaller than the protein coded for by segment 9 (precursor to VP6). In this study, segment 10 produced only one protein which is in contrast to the results of Mertens et al. (1984) with BTV type 1, but similar to the results of Grubman et al. (1983) with BTV type 17. In addition, we did not see a crossover in relative sizes of proteins coded by segments 2 and 3 as observed with BTV type 17 (Grubman et al., 1983).

VP2 of EHDV appears to contain serotype-specific antigenic determinants (Huismans & Erasmus, 1981) while other viral proteins, such as VP3, contain antigenic determinants which are shared with members of the bluetongue group (Huismans et al., 1979). Determination of the protein coding assignment of the genome segments, as reported in this study, will be of value in producing reagents, such as cDNA probes, for the diagnosis of EHDV infections.

REFERENCES


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