Sequence conservation of the outer capsid protein, VP5, of bluetongue virus, a contrasting feature to the outer capsid protein VP2

S. Oldfield, T. Hirasawa and P. Roy

NERC Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford OX1 3SR, U.K. and Department of Environmental Health Sciences, School of Public Health, University of Alabama, Birmingham, Alabama 35294, U.S.A.

The complete nucleotide sequence of a cDNA clone representing the segment 5 RNA of bluetongue virus (BTV) United States serotype 13 was determined. The comparison of the predicted amino acid sequence of the encoded protein (VP5) with the sequences of VP5 from two BTV United States serotypes, 10 and 2, and two isolates of BTV serotype 1 (Australian and South African), revealed that the protein is highly conserved among the different serotypes despite their geographical separation.

Bluetongue virus (BTV) is a dsRNA virus that is an economically important pathogen of sheep and cattle in many parts of the world. The genome consists of 10 dsRNA segments (Verwoerd et al., 1970), each of which encodes a single protein (Mertens et al., 1984). The virion consists of a core of icosahedral symmetry composed of two major proteins (VP3 and VP7) enclosing three minor proteins (VP1, VP4 and VP6), together with the 10 RNA segments (Verwoerd et al., 1972). This core particle is surrounded by a diffuse outer layer of two further proteins (VP2 and VP5). Three non-structural proteins are also synthesized in infected cells but these are not incorporated into the virion (Huismans, 1979).

At least 24 BTV serotypes have been identified (Gorman et al., 1983; Knudson & Shope, 1985) and VP2 has been shown to be the major serotype-specific antigen (Kahlon et al., 1983; Huisman & Erasmus, 1981). Furthermore, VP2 induces neutralizing antibodies which protect sheep against infection with BTV (Huismans et al., 1983; Roy et al., 1990b). However, Wade-Evans et al. (1988) have shown that VP5 can also play a role in the determination of serotype and there is a suggestion that immunization with soluble VP2 and VP5 can induce higher titres of neutralizing antibody than immunization with VP2 alone (Huismans et al., 1983; Roy et al., 1990b).

A comparison of the nucleotide sequences of the VP2 and VP5 genes of various BTV serotypes should indicate the possible serotype-specific regions of the encoded proteins as well as the regions that are conserved, and hence likely to be required for the maintenance of virus morphology; such a comparison should also shed light on the evolution of the various BTV serotypes. We have therefore determined the sequences of genes L2 and M5, encoding the two outer capsid proteins, of different BTV serotypes and analysed the evolutionary relationships of these viruses. Sequence analysis of six VP2 proteins has revealed that the protein is highly variable at the primary structural level, with a number of distinct variable regions (Roy et al., 1990a). In contrast, the VP5 protein appears to be much more conserved and the distinct variable regions are not easily discernible. Since the USA serotype 13 (BTV-13) is serologically more distinct than the three other USA BTV serotypes (namely BTV-10, -11 and -17), and because the VP2 protein of BTV-13 has very little identity with those of these three US serotypes, it was of interest to determine whether the second outer capsid protein, VP5, of BTV-13 behaved similarly (Fukusho et al., 1987). In this report we present the complete nucleotide sequence of a cDNA clone of RNA segment M5 of BTV-13 and compare it with the VP5 nucleotide sequences of various other BTV serotypes.

cDNA corresponding to the full length of segment 5 RNA of BTV-13 was prepared and cloned into pBR322 as described previously (Purdy et al., 1984). One clone, designated pBR13-5, was selected. The insert was excised from pBR13-5 with SacI and treated with exonuclease Bal 31. The resultant shortened molecules were cloned into the SalI site of pUC4K. This generated clones that had lost various lengths of sequence from one or both ends of the segment 5 gene. These were analysed by restriction mapping and a set of overlapping clones was sequenced at both ends using the dideoxynucleotide method (Sanger et al., 1977) with universal and reverse M13 primers. This gave the complete sequence of the gene with the exception of a gap of approximately 50 bp.
in the region between nucleotides 1040 and 1090 and a sequence of eight bp around position 140, which was poorly resolved. The latter sequence was confirmed by sequencing the 5'-terminal HaeIII fragment by the method of Maxam & Gilbert (1980). To complete the sequence, segment 5 DNA was digested with Mael and the smallest fragment was cloned into pUC4K and sequenced as above. The entire sequence is shown in Fig. 1; it is 1637 bp long, one nucleotide shorter than that of the previously reported M5 gene of BTV-10 (1638 bp; Purdy et al., 1986) and two nucleotides longer than that of BTV-2 (Hirasawa & Roy, 1990) and the two isolates of BTV-10 and -2; the terminal non-coding regions are also comparable. The M5 gene of BTV-13 has non-coding sequences of 28 nucleotides at the 5' terminus and 31 nucleotides at the 3' terminus.

The derived amino acid sequence of VP5 of BTV-13 was then aligned with those of BTV-10 and -2 and BTV-1 Australian and South African isolates (Fig. 2). The close similarities between the VP5 sequences of different serotypes were indicated by the fact that no gap was required for maximum alignment. The sequence of BTV-

Fig. 1. Nucleotide sequence of the complete M5 cDNA of BTV-13 and encoded amino acid sequences for the largest open reading frame. Asterisk indicates termination codon.

Fig. 2. Alignment of the predicted VP5 amino acid sequences of five serotypes of BTV. Residues identical to those of BTV-10 are indicated by asterisks.
Table 1. Identity among five BTV VP5 proteins

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<th>BTV-1 (AUS)</th>
<th>BTV-1 (SA)</th>
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<td>BTV-13 (USA)</td>
<td>76</td>
<td>73</td>
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* Percentage of VP5 amino acid sequence identity between BTV serotypes was scored using a computer alignment program.
† Serotypes were isolated in Australia (AUS), South Africa (SA) and the U.S.A.

13 is closest to that of BTV-10, with 82% identity compared to 76% with BTV-2 and 73% and 76% with South African and Australian isolates of BTV-1, respectively (Table 1). The greater identity of VP5 from BTV-13 with that from BTV-10 than with any other VP5 sequenced to date is in contrast to VP2 of BTV-13, which has greater identity to BTV-2 (47%-2%) than to BTV-10 (32%) (Roy et al., 1990a). BTV-2 VP5 and that of both isolates of BTV-1 are more closely related to each other than to BTV-10 and BTV-13. It is interesting to note that the VP5s from both BTV-1 isolates have greater identity with the VP5 of BTV-2 (94%) than they do with each other (88%). It may be that these two isolates have evolved independently from BTV-2 or a closely related BTV serotype. It is obvious that the sequence identity does not appear to reflect the geographical separation of isolates.

Overall, the VP5s of all four serotypes are very closely related, with identities of over 70%. This is in contrast to the VP2s, which are much more diverse with identities ranging between 30 and 60%. The VP2s of BTV-13 and BTV-10 are only distantly related, with an identity of 32% (Roy et al., 1990a) compared to 82% between the VP5s of the same serotypes. It is therefore tempting to speculate that the protein is mainly unexposed on the virus surface.

Inclusion of BTV-13 VP5 in a comparison of derived amino acid sequences with the known primary structures of VP5 of a further three serotypes (including two isolates of one serotype) gives further weight to the assignment of conserved and variable regions. The N-terminal 79 amino acids, C-terminal 45 amino acids and one central region (residues 200 to 270) are highly conserved and may be required for interaction with the core particle and maintenance of virus structure. Other regions, in particular the regions between residues 130 and 190, and 270 and 340, are far more variable, are unlikely to interact with the core particle and may be exposed on the surface of the virion. However, as VP2 is so highly variable between serotypes (Roy et al., 1990a), the minor changes in VP5 may be due to the necessity for it to coevolve with VP2 to accommodate structural changes in that protein.

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References


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