Major core protein VP7 of Australian bluetongue virus serotype 15: sequence and antigenicity divergence from other BTV serotypes

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Full-length cDNA of the RNA genome segment coding for the major core protein VP7 of Australian bluetongue virus serotype 15 (BTV-15) has been isolated by reverse transcription–PCR cloning. Comparative analysis indicated that the BTV-15 VP7 sequence had diverged significantly from that of other members of the BTV serogroup. At the amino acid level, BTV-15 VP7 exhibited sequence identities of 80 to 84 % with VP7 molecules of other serotypes, significantly lower than the sequence identities of between 93 and 100 % observed among other serotypes characterized to date. This was consistent with previous observations that there were significant immunological differences between BTV-15 and other BTV serotypes and that monoclonal antibodies raised against BTV-1 VP7 failed to react with BTV-15 VP7. Recombinant BTV-15 VP7 protein produced from Escherichia coli was largely insoluble, but maintained its immunogenicity. Polyclonal mouse sera raised against the recombinant VP7 protein reacted strongly with VP7 of BTV-15, but weakly with that of BTV-1.

Bluetongue virus (BTV) is the prototype of the Orbivirus genus in the Reoviridae family. The Orbivirus genus is divided into 13 serogroups based on serogroup-reactive procedures such as agar gel diffusion precipitin (Jochim & Chow, 1969) and complement fixation tests (Boulanger & Frank, 1975). Within the BTV serogroup, serological neutralization tests have led to the recognition of at least 24 serotypes.

BTV is a double-shelled virus with a genome consisting of 10 dsRNA segments. The virus has an icosahedral core that contains two major (VP7 and VP3) and three minor (VP1, VP4 and VP6) structural proteins. VP7 is a dominant BTV serogroup-reactive antigen (Huismans & Erasmus, 1981) and is the major, if not sole, constituent of capsomers on the surface of core particles (Hyatt & Eaton, 1988). The core is surrounded by an outer layer composed of two structural proteins, VP2 and VP5. In addition to the seven structural proteins, BTV-infected cells contain three virus-specific, non-structural proteins (NS1, NS2 and NS3).

Recent molecular biology studies indicated that the gene coding for the major serotype-determining protein VP2 varies amongst different serotypes, whereas high sequence identity was maintained for the two major core proteins VP3 and VP7 (Gould & Pritchard, 1991; Eaton et al., 1991; Kowalik & Li, 1991; Wade-Evans, 1990).

Phylogenetic analysis based on VP3 sequences of different BTV serotypes has indicated that evolution of BTV serotypes appears to correspond closely to geographical origin with the exception of the Australian serotype BTV-15 (Gould, 1987; Gould & Pritchard, 1991). This evolutionary divergence of BTV-15 with respect to other members of the BTV serogroup was observed previously in serological tests (Lunt et al., 1987; Della-Porta et al., 1983). More recently it was found that most, if not all, anti-VP7 monoclonal antibodies (MAbs) raised against Australian BTV-1 failed to react with Australian BTV-15 antigens (White, 1992). Competition ELISA (C-ELISA) using either viral VP7 or recombinant VP7 protein, and VP7-specific MAbs, have been developed and shown to be highly effective in the specific detection of anti-BTV antibodies in sera (Lunt et al., 1988; Martyn et al., 1990; Anderson, 1984; Afşhar et al., 1987, 1992). However a number of these tests suffer from a relatively low efficiency of detection of anti-BTV-15 antibodies.

In this study, the complete genome segment coding for Australian BTV-15 VP7 was cloned by reverse transcription–PCR using primers designed according to near-perfect consensus sequences derived from published data. The upstream primer PVP7-5 (5' GTTAAA AATCT MTAGA GATG 3', M: equal molar mixture of
A and C) anneals at the 5' end, whereas the downstream primer PVP7-3 (5' GTAAG TNTAA TCCNA GAG 3', N: equal molar mixture of A, C, G and T) anneals at the 3' end. Each primer has degenerate nucleotides incorporated to accommodate the sequence variations observed in published sequences (Kowalik & Li, 1991; Wade-Evans, 1990; Eaton et al., 1991). For cDNA synthesis, total viral RNA was purified from a plaque-purified BTV-15 virus preparation. The first-strand cDNA was synthesized using primer PVP7-3 following procedures described before (Gould, 1987), and subsequently amplified using primers PVP7-5 and PVP7-3 for 30 cycles at 94 °C for 1 min, 37 °C for 2 min and 72 °C for 2 min. After PCR amplification a major PCR band was observed at 1.1 kb which matched the predicted size. This 1.1 kb PCR fragment was excised from an agarose gel and purified, before being cloned into pUC18 by blunt-end ligation. Three positive clones with inserts of approximately 1.1 kb were obtained, and were subject to double-stranded plasmid DNA sequencing using the USB Sequenase Version 2.0 Kit. Each DNA strand was read at least three times. The sequencing results revealed that the cDNA inserts contained a single open reading frame encoding 349 amino acids, which was highly similar to other published BTV VP7 sequences (see Fig. 1 for amino acid sequence comparison). The full-length nucleotide sequence of the BTV-15AU VP7 gene has been deposited in GenBank.

Amino acid sequence comparison of all the published BTV VP7 sequences in Fig. 1 and Table 1 illustrated several important points. (i) VP7 of BTV-15 exhibited sequence identities of 80 to 84% compared to those of other members of the serogroup and this figure is much lower than the sequence identities (93 to 100%) exhibited among VP7 molecules of other BTV serotypes. This sequence divergence is also reflected at the nucleic acid level as shown in Table 1. There was a nucleotide sequence difference of 27 to 30% between VP7-coding sequences of BTV-15 and all the other BTV serotypes characterized so far. This was significant considering that a much lower degree of divergence of BTV-15 had been observed with respect to the other major core protein VP3. There was only about 4% sequence divergence at the amino acid level and 20% at the nucleic acid level between VP3 molecules of BTV-15 and other BTV serotypes (Gould, 1987; Gould & Pritchard, 1991). (ii) Four highly conserved residues, Cys-15, Cys-65, Cys-154 and Lys-255, in all of the other BTV VP7 sequences in Fig. 1 and Table 1 illustrated that the presence of additional Lys residues in VP7 of BTV-15 will have any effect in its interaction with VP3 of a different serotype during the formation of virus core particles. (iii) There was a Thr to Ala change in the highly conserved sequence of six amino acids constituting epitope QYPALT (positions 259 to 264), which was recognized by two VP7 specific MAbs (du Plessis et al.,

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Fig. 1. Alignment of the deduced VP7 amino acid sequences of different BTV serotypes. US, U.S. serotypes (Kowalik & Li, 1991); SA, South African serotypes (Wade-Evans, 1990); AU, Australian serotypes (Eaton et al., 1991). Identical amino acid residues are indicated by dots whereas residues different from those of BTV-2US are shown by the one-letter amino acid code. The solid arrowheads (▼) indicate Cys and Lys residues conserved in all BTV VP7 proteins and the open arrowheads (▲) indicate Cys and Lys residues unique to BTV-15AU. The six amino acid epitope (positions 259 to 264) which was recognized by two VP7 specific MAbs (du Plessis et al., 1994) is indicated by the bracket above the BTV-2US sequence. The sequence alignment was done using the AlignPlus program from Scientific & Educational Software.
Table 1. Identity (%) of amino acid (nucleotide) sequences of VP7 among eight BTV serotypes

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1994). (iv) The most highly conserved domain in all BTV VP7 molecules, including BTV-15 VP7, was located at the N terminus between residues 28 and 54. This region seems to be highly conserved in VP7 from other Culicoides-borne but not tick-borne orbiviruses (Roy et al., 1991; Iwata et al., 1992; Moss et al., 1992). Recent studies have revealed that although core particles of BTV show a large reduction in their infectivity for vertebrate cells compared with intact viruses, they remain infectious to insect cells to the same degree as complete virus particles (Mellor, 1990). It is therefore tempting to speculate that the highly conserved region of VP7, in a region of the molecule known to be exposed on the surface of virus and core particles (Eaton et al., 1991; L.-F. Wang, unpublished), may play a role in the interaction between core particles and receptors on the surface of insect cells.

The other difference at the gene level was the length of the 3' non-coding region of BTV-15 VP7, which was four nucleotides shorter than the corresponding regions of the five BTV serotypes and one South African serotype (Kowalik and Li, 1991; Wade-Evans, 1990), and two nucleotides shorter than that of Australian BTV-1 (Eaton et al., 1991). The biological significance of this difference in the 3' non-coding region is not clear. There was no difference between the 5' non-coding region of BTV-15 and that of other BTV serotypes.

One of our purposes in cloning the BTV-15 VP7 gene was to obtain recombinant protein for immunological studies of VP7 molecules. This was achieved by expression of the cDNA clone in Escherichia coli using the pET expression plasmid, a T7 RNA polymerase-directed expression system developed by Studier et al. (1990). For this, the coding region of BTV-15 VP7 was modified by PCR-mediated mutagenesis. A BamHI site was introduced upstream of the ATG start codon using primer P181 (5' CCCGG GATCC AGAGA TGGAC ACTAT CTGCA 3'), and primer P528 (5' GTCAG AATTC CGTCG ACCGA CACTC AGGTA T 3') was used to introduce an EcoRI site downstream from the stop codon TAG. The resulting BamHI–EcoRI fragment was inserted into the expression vector pET-5b to form pET-15VP7. After the plasmid had been introduced into E. coli host strain BL21(DE3), expression was induced by the addition of IPTG (Studier et al., 1990). The expression results are presented in Fig. 2(a). A strong band corresponding to approximately 36K to 38K was present in the pellet fraction of total lysate from cells containing pET-15VP7, but absent from the control culture containing the vector pET-5b alone. These results demonstrated that a specific gene product had been expressed from the cloned cDNA insert and that the recombinant VP7 proteins expressed were largely insoluble. A similar observation was made when the VP7 gene was expressed from the pGEX vector (Smith & Johnson,
molecule, which leads to a high probability of affecting most, if not all, of a panel of well characterized MAbs cross-react with BTV-15 VP7 (White, 1992). The in amino acid residues are scattered along the entire molecular evidence for this observation. Although there tested for their reactivity using purified BTV-15AU and BTV-1AU proteins has been observed before for VP7 of BTV-15 VP7 molecules. This confirmed that the recombinant VP7 expressed from pET-15VP7 was antigenically different from the BTV-1 VP7 protein.

Recent studies from this laboratory indicated that most, if not all, of a panel of well characterized MAbs raised against BTV-1 VP7 did not show any significant cross-reaction with BTV-15 VP7 (White, 1992). The sequence comparison presented in Fig. 1 provides the molecular evidence for this observation. Although there are sequence identities of 80 to 84% between VP7 of BTV-15 and those of other BTV serotypes, the differences in amino acid residues are scattered along the entire molecule, which leads to a high probability of affecting MAb-specific epitopes at any region in the molecule. This has already been demonstrated for the 20D11/20F10-specific epitope (see Fig. 1 and du Plessis et al., 1994), where a single change of Thr to Ala in BTV-15 VP7 prevented the MAbs binding to a well defined six amino acid epitope (Fig. 2b).

Using the recombinant BTV-15 VP7 protein readily available from bacterial expression, we also investigated whether antibodies raised against denatured recombinant BTV-15 VP7 would be able to react with viral VP7 proteins of different serotypes. For this, the insoluble VP7 protein expressed from pET-15VP7 in E. coli was purified by preparative SDS–PAGE as described by Hager & Burgess, 1980). Approximately 5 μg of purified recombinant protein in a total volume of 75 μl was emulsified with an equal volume of incomplete Freund's adjuvant, and inoculated intramuscularly into four BALB/c mice. Booster inoculations, with and without incomplete Freund's adjuvant, were given intramuscularly at 3-week intervals. Antisera were collected 4 weeks after the third injection and were analysed by ELISA for reactivity against viral antigens. The results in Fig. 3 show that although there was some variation between different mice the bacterial recombinant protein was able to stimulate a specific immune response against viral VP7 proteins. The antibodies raised against the denatured recombinant BVT-15 VP7 protein reacted strongly with BTV-15 viral antigen. However the reaction with BTV-1 antigen was much weaker. These results confirmed the general observation that Australian BTV-15 was antigenically distant from other Australian BTV serotypes. On the other hand, the results also suggested that although weak, a specific cross-reaction did occur between VP7 proteins of BTV-15 and BTV-1, which varied in degree from animal to animal. We are currently investigating the possibility of raising MAbs or polyclonal antibodies which are truly serogroup-specific, i.e. antibodies that will recognize VP7 proteins of all BTV serotypes including Australian BTV-15. Such genuine serogroup-specific antibodies may enable us to develop an improved C-ELISA that will detect BTV-15 antibodies more efficiently than existing tests.

In summary, the results presented here demonstrated that Australian BTV-15 VP7 is significantly different from that of other BTV serotypes both in sequence and in antigenicity. This not only raised an interesting question with respect to the evolution of BTV-15 in relation to other serotypes, but also provided useful information for future development of improved ELISAs which could efficiently detect anti-BTV-15 antibodies. VP7 is not only a major group-reactive antigen and a good stimulator of the B cell-mediated immune response, but the presence of cross-reactive cytotoxic T cells in sheep suggests that a conserved major viral protein such as VP7 may also contain T cell epitopes (Jeggo & Wardley, 1985; Jeggo et al., 1984). Therefore a better understanding of the molecular and immunogenic properties of VP7 from antigenically divergent BTV serotypes.
may help in the logical development of potential vaccines against infection by different BTV serotypes. Also, the sequence and antigenic properties of BTV-15 VP7 have proved to be extremely useful in distinguishing between MAb-defined VP7 epitopes that are different between BTV-15 and other BTV serotypes (du Plessis et al., 1994; L.-F. Wang, unpublished).

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References


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