Phylogenetic relationships of bluetongue viruses based on gene S7

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Abstract

Previous phylogenetic analyses based on bluetongue virus (BTV) gene segment L3, which encodes the inner core protein, VP3, indicated a geographical distribution of different genotypes. The inner core protein, VP7, of BTV has been identified as a viral attachment protein for insect cell infection. Because the inner core proteins are involved with infectivity of insect cells, we hypothesized that certain VP7 protein sequences are preferred by the insect vector species present in specific geographic locations. We compared the gene segment S7, which encodes VP7, from 39 strains of BTV isolated from Central America, the Caribbean Basin, the United States, South Africa and Australia. For comparison, the S7 sequences from strains of the related orbiviruses, epizootic hemorrhagic disease virus (EHDV) and African horse sickness virus (AHSV) were included. The S7 gene was highly conserved among BTV strains and fairly conserved among the other orbiviruses examined. VP7 sequence alignment suggests that the BTV receptor-binding site in the insect is also conserved. Phylogenetic analyses revealed that the BTV S7 nucleotide sequences do not unequivocally display geographic distribution. The BTV strains can be separated into five clades based on the deduced VP7 amino acid sequence alignment and phylogeny but evidence for preferential selection by available gnat species for a particular VP7 clade is inconclusive. Differences between clades indicate allowable variation of the VP7 binding protein. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Phylogenetic relationships; Bluetongue viruses; Gene S7

1. Introduction

Bluetongue virus (BTV) is an economically important insect-transmitted orbivirus (Family: Reoviridae) that causes disease in domestic and wild
ruminants. Other closely-related orbiviruses are epizootic hemorrhagic disease virus (EHDV) of
deer and African horse sickness virus (AHSV).
Biting gnats of the Culicoides species transmit
these viruses. The primary insect vector species
for BTV in North America is Culicoides sonorensis
(Tabachnick, 1996; Holbrook et al., 2000); whereas, Culicoides insignus is considered the
common vector in Central America and the
Caribbean Basin (Mo et al., 1994). Although
there are a number of Culicoides species in South
Africa the only proven vector species are Culico-
oides bolitinos and Culicoides imicola (Venter et
al., 1998). In Australia, Culicoides brevitaris, Culicoides wadai, Culicoides fulus, and Culicoides
actoni have been associated with BTV transmis-
sion (St. George, 1985; Standfast and Muller,
1989). Different serotypes of BTV are associated
with different geographic regions. In the United
States, five BTV serotypes (2, 10, 11, 13 and 17)
have been isolated; whereas, serotypes 1, 3, 4, 6, 8,
12 and 17 have been isolated in Central America
and the Caribbean Basin (Mo et al., 1994). Serotype 17 is the only serotype common to the
US and the Caribbean Basin with serotypes 1, 3
and 4 established both in South Africa and the
Caribbean Basin. Bluetongue virus serotype 2 has
been isolated in South Africa and the Southeast-
ern United States but not in the Caribbean Basin.
The presence of different serotypes and vector
species in distinct geographic areas suggests there
may be a relationship between virus strains and
competent insect vector species.
Investigations of the molecular interactions of
these viruses with their insect vector hosts have
shown that VP7 is involved with binding to insect
membrane proteins (Xu et al., 1997). Although
VP7 is an inner core protein, it is accessible on the
outer surface of intact virus particles (Lewis and
Grubman, 1990; Eaton et al., 1991). It has been
demonstrated that BTV core particles with an
exposed inner shell, consisting of VP3 and VP7,
are as infectious to vector insects as intact virus
particles with an outer capsid containing VP2 and
VP5 (Mertens et al., 1996). In mammalian cells,
however, core particles that lack VP2 and VP5
have reduced infectivity compared to intact
viruses (Mertens et al., 1996). This phenomenon
indicates that VP3 and VP7 are important in the
ability of BTV to infect the insect vector. Both of
these proteins are relatively conserved, and VP3
has distinct genotypes in different geographical
areas (Pritchard et al., 1995). This study examines
whether the S7 gene that encodes VP7 also dis-
plays distinct genotypes in different geographical
areas that could be related to vector competence.

2. Materials and methods

2.1. Viruses and purification of dsRNA

Isolates of BTV exotic to the US were obtained
from the Inter-American Bluetongue Project (Mo
et al., 1994) and from the Onderstepoort Veterinary
Institute virus library. The United States
BTV and EHDV isolates were obtained from the
Arthropod-Borne Animal Diseases Research Lab-
oratory (ABADRL) reference collection. Table 1
lists the passage histories and virus strains used in
this study. Sequences obtained from the literature
are referenced. The dsRNA template was pre-
pared using a differential lithium chloride precipi-
tation procedure as described previously (Wilson
et al., 1990). The S7 from selected strains of BTV
serotypes 1, 3, 4, 6, 8 and 12 isolated from the
Caribbean Basin and serotypes 1, 2, 3 and 4
isolated from South Africa were sequenced. In
addition, two virus strains of BTV serotype 2 with
distinct dsRNA electrophoretic patterns desig-
nated as Ona A and Ona B isolated from Ona,
Florida (Collisson et al., 1985) were sequenced.
For comparisons between related orbiviruses, the
sequence of S7 from the prototype North Ameri-
can strain of EHDV serotype 2 was also
determined.

2.2. Cloning and sequencing

Viral strains were cloned by reverse transcrip-
tase/polymerase chain reaction (RT/PCR) using
terminal primers as described previously (Wilson,
1994). Sequence data were obtained using standard
automated sequencing (Smith et al., 1986; Applied
Biosystems Inc., Foster City, CA) di-
rectly from PCR products and from cloned am-
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Table 1
List of virus strains and Gen Bank accession numbers for L7 sequences
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<td>Wade-Evans et al., 1993</td>
</tr>
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</table>

a Virus passage histories where known. The number prior to the abbreviation is the number of passages. Abbreviations: BHK, baby hamster kidney cells; ECE, embryonated chicken eggs; L929, mouse fibroblast cells; Vero, African green monkey kidney cells.

b Sequences not referenced are new S7 sequences reported in this paper.

c First recorded cell-culture passage.

plification products using the commercially available TA cloning kit (Invitrogen, San Diego, CA). Complete sequences were determined in both the sense and anti-sense directions. When plasmid clones were used to generate sequencing templates, three separate clones were sequenced to correct for any potential sequence errors in a single clone.

2.3. Computer analyses

Sequence data were compiled with SeqMan software (DNA Star Inc., Madison, WI) and analyzed with GCG software programs version 10 (Oxford Molecular Inc., Mountain View, CA). Phylogenetic analyses were performed using the following computer programs: Molecular Evolutionary Genetics Analysis, MEGA, version 1.01 (Kumar et al., 1993), Phylogeny Inference Package, PHYLIP version 3.5 (Felsenstein, 1993) and Phylogenetic Analysis Using Parsimony, PAUP version 4.0b3a (Swofford, 1997).

3. Results and discussion

The BTV S7 genes are 1154–1156 base pairs (bp) in length and have a single open reading frame encoding a predicted protein of 349 amino acids. The EHDV S7 gene is 1161 bp in length and also has a single open reading frame encoding a protein of 349 amino acids. Alignment of the deduced amino acid sequences demonstrates the conservation of this protein sequence. The more variable region that also contains the putative receptor-binding domain and has been shown to be surface accessible is depicted in Fig. 1. With the exception of BTV15 AU, the first 120 amino acid residues are very highly conserved (96.7–99.2% identity). This is probably a result of the amino and carboxy terminal residues involvement in the VP7-VP3 interactions in forming the core particle (Monastryskaya et al., 1997). Previously mapped immunodominant antigenic epitopes of the US serotypes at amino acid residues 122–139 and 11 residues at the carboxyl terminus (Li and Yang, 1990) are relatively conserved (Fig. 1, underlined; 82.4–88.2% and 81.8–90.9%, respectively). A BTV epitope at residues 263–267, that was previously identified as potentially cross-reactive with a related EHDV (Du Plessis et al., 1994), is highly conserved between the BTV serotypes (78.6–100%), EHDV serotypes (100%) and between BTV and EHDV serogroups (64.3%). However, these amino acid sequences are different in another orbivirus, AHSV.

The crystal structure of BTV10 VP7 has been reported and the upper domain is an anti-parallel β-sandwich and the strands common to the jelly-roll motif (Grimes et al., 1995). These common strands, as with the 263–267 epitope, are highly conserved among BTV strains and fairly conserved among EHDV strains. The RGD tripeptide (double underline) responsible for the integrin-dependent cell adhesion processes (Grimes et al., 1995) is conserved among all isolates except for BTV13 US and BTV15 AU. This position for BTV13 US has an isoleucine at the arginine position, which still maintains an integrin-dependent domain sequence. The lysine
found to be critical for inner core structure formation (Le Blois and Roy, 1993) is conserved among all BTV and EHDV serotypes.

Genotypes specific to geographic location were not as clearly defined by phylogenetic analyses based on the complete S7 nucleic acid sequences (Fig. 2) as described previously for the L3 gene (Pritchard et al., 1995). For example, the US strains are primarily in the clade 1 but BTV13 US is in clade 5 containing strains isolated from Central America and the Caribbean Basin. As noted with L3 (Pritchard et al., 1995), the S7 sequences from South African BTV1 strains are more closely related to a strain of BTV1 from
Fig. 2. Phylogenetic analysis of the S7 gene sequence for various orbiviruses. Potential vector species based on isolate origin are listed under clade or clade designation. The numbers indicate bootstrap confidence values after 500 replications. The tree was generated using MEGA and the following analysis parameters: gamma distance measure ($\alpha = 1$), Kimura 2-parameter model and neighbor-joining bootstrap analysis.
interactions of the virus with its receptor. Phylogenetic trees based on the nucleotide coding for this region identified the same genetic relations at similar confidence levels (data not shown). The rates of non-synonymous ($K_a$) and synonymous ($K_s$) changes were calculated (Li, 1993; data not shown). The $K_a$, as expected, was less than the $K_s$ and the trends were similar to that depicted by the phylogenetic analysis. The $K_a/K_s$ data can be used to demonstrate positive selection, however, in this case the results are inconclusive.

Three serotypes are thought to be recent introductions into geographic regions and representative strains of these serotypes were chosen for this study. The most recent serotype to be introduced in the US is BTV2. Early after its introduction, this serotype had an RNA electrophoretic profile similar to BTV2 from South Africa but quickly changed to a new profile that persisted during the outbreak (Collisson et al., 1985). The virus strains with the original profile are called BTV2 OnaA US and the strains with subsequent profile are called BTV2 OnaB US. The coding region S7 of BTV2 OnaA US was determined to be 99.8% identical to BTV2 557 SA and only 79.4% identical to BTV11 US. BTV2 OnaB US, however, was only 79.8% identical to BTV2 557 SA but 92.8% identical to BTV11 US. This data is consistent with the hypothesis that this virus originated from South Africa. This dramatic genetic change could be a result of a reassortment event with indigenous BTV strains or environmental selection of a more favorable genotype. Further study is needed to determine if there is a difference in the infection rate of $C$. sonorensis by these two virus types.

Bluetongue virus serotype 3 was the most recent serotype to be isolated in the Caribbean Basin and Central American region (Mo et al., 1994). The first S7 sequence determined from a BTV3 strain in this region was similar to the BTV3 565 SA sequence. This suggested that BTV3 in this region originated from South Africa. The S7 from several BTV3 strains were sequenced to determine if a genetic pattern of change could be followed as this serotype apparently moved across the region. Also, several strains isolated from Panama were sequenced to determine change over time. The early strains isolated in

Honduras than to the Australian BTV1 strain. The Australian BTV15 remains the most distinct serotype (Pritchard et al., 1995). Fig. 2 was generated using the Kimura two parameter model that assumes that all nucleotide substitutions occur randomly and that transitions are generally more frequent than transversions. The tree was generated using the neighbor-joining method that finds neighbors sequentially to minimize the total length of the tree. Maximum parsimony analysis generates trees that minimize the number of evolutionary changes (Li, 1997). The phylogenetic groupings are similar when the S7 coding region is analyzed with the maximum parsimony method (Fig. 3).

Amino acids that comprise the VP7 top domain (Monastryskaya et al., 1997) are likely involved in
Fig. 4. Phylogenetic analysis of the deduced amino acid sequence for various orbiviruses. Potential vector species based on isolate origin listed under clade or clade designation. The tree was generated using MEGA and the following analysis parameters: Poisson correction and neighbor-joining bootstrap analysis. The numbers indicate bootstrap confidence values after 500 replications.
1988 were all in the nucleic acid phylogenetic clade 6 that included BTV3 565 SA. Two of the five 1989 strains were in clade 6, two in clade 5 and one in clade 1, containing the majority of US strains. The majority of the 1990–1992 BTV3 strains isolated in this region were in clades 5 and 1, with only one, BTV3 2270 ES, remaining in clade 6. Although only a small number of strains were examined, there was a general shift toward a S7 genotype similar to that found in the region.

It is suspected that the BTV17 isolated in Central America and the Caribbean Basin probably came from the US (Mo et al., 1994). The S7 sequence data from a few strains of BTV17 is supportive of this suspicion, as three of the four strains examined were phylogenetically in clade 1, which contains a majority of viruses of US origin. The outlier, BTV17 296 PR, was in clade 6 and may have resulted from reassortment. Alternatively, this virus could also represent a genetic shift towards a S7 genotype similar to that found in the region. Additional isolates after 1992 are not available to determine if these trends continued.

Phylogenetic grouping utilizing deduced amino acid sequences shows a reduction in the distinction of geographic clades (Fig. 4). These findings indicate there is random variation among virus populations resulting in at least five BTV VP7 types that are moderately dependent on geographical location. Four of the five US serotypes are in VP7 clade 1 and BTV13 is found in VP7 clade 4. The BTV13 prototype strain also contained the most distinct VP3 sequence among the US serotypes (Pritchard et al., 1995). The South African, Central American, and Caribbean Basin strains are spread between three VP7 clades. The most diverse are the two published Australian strains that separate into distinct clades. Perhaps the multiple Culicoides species associated with BTV transmission in Australia allow more variation in the VP7 protein. The South African Culicoides species are different from the Central America and Caribbean Basin principle species; however, viruses isolated within these regions are similar. This suggests that the virus-insect receptor interactions are similar.

The correlation between Culicoides species and viruses from particular S7 or VP7 clades is not strong. Neither phylogenetic trees generated using VP7 amino acid sequences in close proximity to the putative RGD integrin binding site nor trees generated using only the VP7 amino acid sequences thought to be accessible to the outer surface (120 residues) strengthened this correlation (data not shown). The lack of relationship may in part be due to use of prototype and cell-culture adapted virus isolates in this study. The lowest passage cell culture-adapted virus strains available were used because the original virus infected tissues were not available for direct PCR. Virus isolation through embryonated chicken egg inoculation (ECE) and cell-culture could select for a particular virus population. Still, differences in infection rates of colonized C. sonorensis have been shown with laboratory BTV US serotype strains (Mecham and Nunamaker, 1994). Also, differences in the infection rates of three Culicoides species by three South African serotypes have been demonstrated (Venter et al., 1998). The mechanistic controls for these differences are likely to be complex. Very little is known about the biochemical requirements for orbivirus infection of its invertebrate host. To understand this interaction, the genetics of the virus and the insect vector populations present needs to be considered. This study begins to define the genetics of one gene that is a component of the complex combination of factors involved in the infection process.

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