Isolation and identification of a new tetravirus from *Dendrolimus punctatus* larvae collected from Yunnan Province, China

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In this study, *Dendrolimus punctatus* tetravirus (DpTV) has been identified as a new member of the genus *Omegatetravirus* of the family *Tetraviridae* that may be related serologically to *Nudaurelia capensis* virus (NoV). DpTV particles are isometric, with a diameter of about 40 nm and a buoyant density of 1.28 g cm\(^{-3}\) in CsCl. The virus has two capsid proteins (of 62 500 and 6800 Da) and two single-stranded RNA molecules (RNA1 and RNA2), which are 5492 and 2490 nt long, respectively. RNA1 has a large open reading frame (ORF) encoding a polypeptide of 180 kDa; RNA2 contains two partially overlapping ORFs encoding polypeptides of 17 and 70 kDa. The 180 kDa protein, which contains consensus motifs of a putative methyltransferase, helicase and RNA-dependent RNA polymerase, shows significant similarity to those of other tetraviruses. The 17 kDa protein is a PEST (Pro/Glu/Ser/Thr) protein of unknown function. The 70 kDa protein is the coat protein precursor and is predicted to be cleaved at an Asn–Phe site located after residue 570. The 70 kDa protein shows 86 and 66 % identity to its homologues in *NoV* and *Helicoverpa armigera stunt virus*, respectively. Secondary-structure analysis revealed that the RNAs of DpTV have tRNA-like structures at their 3' termini.

**INTRODUCTION**

The family *Tetraviridae* comprises small RNA viruses whose host range is restricted to lepidopteran insects (butterflies and moths) (Hanzlik & Gordon, 1997). Viruses in this family have single-stranded, positive-sense RNA genomes encased in unenveloped, icosahedral virions that are 35–41 nm in diameter, with equilibrium densities in CsCl of <1.30 g cm\(^{-3}\). The family now comprises 11 confirmed members, including two viruses whose complete nucleotide sequences have been reported and show different genomic organization and eight unassigned possible members. The family *Tetraviridae* is divided into the genera *Betatetravirus* and *Omegatetravirus* on the basis of capsid appearance and genome organization (Hendry et al., 1995; Hanzlik & Gordon, 1997; Pringle et al., 1999, 2003).

Betatetraviruses, such as *Nudaurelia capensis* \(\beta\) virus (NfV), *Thosea asigna* virus (TaV) and Providence virus (PrV), have a bipartite genome with both the replicase and capsid precursor genes on an RNA molecule of ~6.5 kb. The capsid precursor gene is also present on a subgenomic RNA molecule of ~2.5 kb (Gordon et al., 1999; Pringle et al., 1999, 2003). Omegatetraviruses, such as *Nudaurelia capensis* \(\alpha\) virus (NoV) and *Helicoverpa armigera stunt virus* (HaSV), have a bipartite genome with the replicase and capsid precursor genes on the ~5.3 and ~2.5 kb RNA molecules, respectively (Agrawal & Johnson, 1992; Hanzlik et al., 1993, 1995; Gordon et al., 1995). Capsids of both genera contain two proteins: a major protein of ~60 kDa and a minor protein of ~7 kDa.

The tetraviruses are currently the only known non-enveloped viruses with \(T=4\) icosahedral symmetry. Their structures and their property to undergo maturational cleavage and structure rearrangement have drawn considerable interest to the family in recent years (Hanzlik & Gordon, 1997). The structure of *NoV* has been solved by both X-ray crystallography and electron cryomicroscopy (cryoEM) (Johnson et al., 1994; Munshi et al., 1996, 1998) and that of *NfV* has been examined by transmission electron microscopy and cryoEM (Finch et al., 1974; Olson et al., 1990). Each tetravirus particle is composed of 240 copies of two structural proteins, which form four Y-shaped trimers on each face of the capsid. The two structural proteins are produced by post-assembly cleavage of a single capsid precursor protein (Agrawal & Johnson, 1992, 1995; Hanzlik et al., 1995; Pringle et al., 1999, 2001, 2003). The virion undergoes large conformational changes during this cleavage (Canady et al., 2000, 2001).
Here we report the identification of a small RNA virus isolated from diseased *Dendrolimus punctatus*, a member of the family Lasiocampidae, collected from Yunnan Province, China. In the present study, we have characterized the particle properties of this virus and determined the nucleotide sequences of the genome. The results indicate that it is a new virus that should be classified within the genus *Omegatetravirus* of the family *Tetraviridae* and we have tentatively named it *Dendrolimus punctatus* tetravirus (DpTV).

**METHODS**

Sources of standard viruses and insect cadavers used for virus isolation. NpV and NoV, and antisera to NpV and NoV, were supplied by D. Hendry (Rhodes University, South Africa). HaSV and antiserum to HaSV were supplied by T. N. Hanzlik (CSIRO Entomology, Australia). Antiserum to TaV was supplied by V. K. Ward (University of Otago, New Zealand). Infected *D. punctatus* larvae were collected from the field in Yunnan Province, China, and were either used immediately or kept frozen at −20°C prior to processing for virus purification.

Virus purification from frozen *D. punctatus* larvae. Frozen insect larvae (200 g) were thawed and homogenized in 500 ml extraction buffer [0·05 M Tris/HCl, 0·001 M EDTA (pH 7·5), 0·2 % 2-mercaptoethanol]. The homogenate was clarified by centrifugation at 10,000 g for 30 min. The supernatant was precipitated by centrifuging at 100,000 g for 2 h in a Beckman Coulter Ti70 rotor. Virus pellets were resuspended overnight at 4°C in TE buffer [0·05 M Tris/HCl, 0·001 M EDTA (pH 7·5)]. The resuspended virus was centrifuged through a 30 % (w/v) sucrose and pelleted at 100,000 g for 4 h at 100,000 rpm; then layered on a 10–40 % (w/v) sucrose gradient and centrifuged at 100,000 rpm in a Biofuge Stratos rotor for 30 min. The supernatant was precipitated by centrifuging at 100,000 g for 2 h in a Beckman Coulter Ti70 rotor. Virus pellets were resuspended overnight at 4°C in TE buffer [0·05 M Tris/HCl, 0·001 M EDTA (pH 7·5)]. The resuspended virus was centrifuged through a 30 % (w/v) sucrose cushion at 100,000 g for 3 h. Virus pellets were resuspended again, then layered on a 10–40 % (w/v) sucrose gradient and centrifuged at 100,000 g for 2 h in a Beckman Coulter Ti40 rotor. The band containing virus particles was collected, diluted with TE buffer and pelleted at 100,000 g for 3 h. Virus pellets were resuspended in 250 μl TE buffer and frozen for long-term storage. For further purification, virus in TE buffer was subjected to a CsCl density gradient that was equilibrated by centrifugation at 300,000 g for 14 h at 4°C in a Hitachi CP100MX P100 AT2 rotor. The opalescent band of virus was removed from the gradient and pelleted at 100,000 g for 3 h in TE buffer. Buoyant density was determined in CsCl gradients according to Scotti (1985). The purity and integrity of the purified virus were verified by negative staining with 2 % (w/v) phosphotungstic acid (PTA, pH 7·0) and examined under a Hitachi H-8100 transmission electron microscope.

Protein characterization. The capsid proteins of DpTV were analysed by SDS-PAGE. Samples were boiled in 2 x sample buffer for 5 min before electrophoresis through a 12 % glycine SDS-PAGE gel (Laemmli, 1970). Samples of NoV and HaSV were also analysed on the same gel as the control. Proteins were stained with Coomassie brilliant blue R-250. A middle-range molecular mass marker was included as a size standard.

To determine whether DpTV has the 7 kDa protein that is shared by other tetraviruses, Tricine SDS-PAGE was used as described by Schagger & von Jagow (1987), in conjunction with a broad-range, pre-stained protein marker.

Nucleic acid characterization. RNA was extracted from capsids by using TRizol reagent according to the manufacturer’s instructions (Invitrogen), except for the addition of 1 μl glycerol (20 mg ml⁻¹) to enhance RNA precipitation. The sample was subjected to electrophoresis in a 1 % agarose gel containing formaldehyde (Sambrook *et al.*, 1989). Nucleic acids were digested with RNase A or DNase I, using single- and double-stranded DNA and single-stranded RNA as controls, then subjected to electrophoresis.

Immunological procedures. The serological relationships between DpTV and other known tetraviruses, including NpV, NoV, HaSV and TaV, were determined by Western blot immunoassay as described by Towbin *et al.* (1979). Antiser to the viruses were used as positive controls (except for TaV, due to unavailability of the virus).

cDNA synthesis and cloning. cDNA fragments complementary to DpTV RNA were synthesized in the presence of random primers, dNTPs and Thermoscript reverse transcriptase (Invitrogen), as recommended by the manufacturer. The cDNA was blunt-ended with T4 DNA polymerase, size-selected for lengths >400 bp on a spin column and ligated into the *Sma*I site of pUC18. The ligation mixture was transformed into *Escherichia coli* DH5α cells by electroporation.

Some clones were generated by PCR to cover missing sequences, using primers based on the flanking sequences available from the cDNA clones. The rapid amplification of cDNA ends (RACE) method was used to determine the complete nucleotide sequences of the 5’ and 3’ termini of DpTV RNAs (Iwamoto *et al.*, 2001). For 5’ RACE, the first cDNAs were polyadenylated with terminal deoxynucleotidyl transferase. For 3’ RACE, viral RNA was polyadenylated with poly(A) polymerase (Invitrogen) in the presence of ATP. The amplified product was inserted into the pMD18 vector.

Nucleotide sequencing and sequence analysis. Both strands of the plasmids obtained were sequenced by the dideoxynucleotide chain-termination method, using an ABI Prism 377 DNA sequencer (Applied Biosystems) with universal sequencing and walking primers.

Open reading frames (ORFs) were identified by using BLAST (http://www.ncbi.nlm.nih.gov/blast/). Sequence homology was obtained by using the DNAMAN program (Lynnon Corporation). The conserved sequences were aligned by using CLUSTAL_W version 1.8. Phylogenetic and molecular evolutionary analyses were conducted by using MEGA version 2.1 (Kumar *et al.*, 2001) with the neighbour-joining algorithm. RNA secondary structure was analysed by the Mfold program online (http://www.bioinfo.rpi.edu/applications/mfold) and by STAR version 4 (Abrahams *et al.*, 1990; Gultyaev, 1991).

**RESULTS**

Characterization of DpTV particles

The buoyant density of DpTV particles in CsCl at pH 7·5 was 1·281 g cm⁻³. When negatively stained with PTA, they appeared isometric and non-enveloped, had a mean diameter of about 40 nm and displayed an external ring around a central core, similar to the structure reported for several other insect RNA viruses (Tinsley & Melnick, 1973; Bailey, 1976; Morris *et al.*, 1979). The stain accumulated along the fivefold vertices of the virus, suggesting a triangulation number of 4 or 9 (Morris *et al.*, 1979) (Fig. 1). This indicated that this virus might be a member of the family *Tetraviridae*.

Coat protein and nucleic acid characterization

Examination of DpTV capsid proteins by SDS-PAGE revealed that they are similar, but not identical, to those of NoV (Fig. 2a, b). DpTV has two capsid proteins: a major
protein of 62,500 Da and a minor protein of about 6,800 Da (Fig. 2a, b). The molecular mass of the DpTV major protein was lower than that of HaSV, but slightly higher than that of NωV; the molecular mass of the DpTV minor protein was lower than those of both HaSV (7,309 Da) and NωV (7,817 Da) (Hanzlík & Gordon, 1997). The relative molarities of each protein were comparable.

Nucleic acids extracted from DpTV particles were hydrolysed readily by RNase A, but not by DNase I, under similar conditions, indicating that they are single-stranded RNAs. The RNA preparation migrated as two species (RNA1 and RNA2) in agarose gel under denaturing conditions; the sizes of these were estimated as approximately 5,500 and 2,500 nt, although there was some degradation (Fig. 2c).

Serological relationship of DpTV with other tetraviruses

A Western blot immunoassay showed that DpTV reacted with antisera to NωV (Fig. 3), but not with antisera to HaSV, NβV or TaV (data not shown). This indicates that DpTV may be related serologically to NωV, the prototypical member of the genus Omegatetravirus. It should be noted that no TaV was available as a positive control in these experiments.

Nucleotide sequences and genomic organization

We determined the nucleotide sequences of DpTV RNA1 and RNA2. RNA1 consisted of 5,492 nt and had a G+C content of 57.3 mol% (20.3 mol% A, 22.5 mol% T, 31.5 mol% C, 25.8 mol% G). Sequence analysis identified a large ORF encoding 1,649 aa, starting with the first AUG (nt 37–39) and terminating at UGA (nt 4,984–4,986). This protein, named p180, had a calculated molecular mass of 179,474 Da and a calculated pl of 8.10. The next longest ORF on RNA1, which started at nt 4,491 and ended with

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**Fig. 1.** Transmission electron micrograph of negative-stained virions. Black arrowheads indicate fivefold vertices; white arrowheads indicate central cores into which stain penetrated.

**Fig. 2.** Characterization of DpTV proteins and RNA. (a) Identification of 62.5 kDa DpTV capsid proteins by 12% glycine SDS-PAGE. HaSV and NωV are shown for comparison. MI, Middle-range marker. (b) Identification of 62.5 and 6.8 kDa capsid proteins of DpTV by 16.5% Tricine SDS-PAGE. MII, Broad-range, pre-stained marker. (c) Gel-electrophoretic analysis of RNA extracted from DpTV particles. M, 1 kb RNA marker; 1, DpTV RNA.

**Fig. 3.** Western blot immunoassay for cross-reactivity between DpTV and antiserum to NωV. 1, NωV, used as positive control; lane 2, DpTV. Markers in kDa are shown on the left.
a UGA stop codon at nt 4898, encoded a protein of 135 aa, named p15. ORFp15 was flanked by shorter ORFs of 118 aa (nt 4997–5353, encoding p13) and 98 aa (nt 4194–4490, encoding p11). The UAG termination codon at the end of the putative ORFp11 was located immediately to the 5’ side of ORFp15, theoretically allowing these two ORFs to form a single 230 aa protein if the stop codon were suppressed. Expression of these short ORFs would almost certainly require a subgenomic mRNA, because of the 5’ long leading sequence. The nucleotide sequence of DpTV RNA1 was 61 % identical to that of HaSV, although the HaSV RNA1 sequence is 180 nt shorter. Notably, the 3’ non-coding sequence of DpTV RNA1 was the same length as that of HaSV (36 nt).

RNA2 was 2490 nt in length and was cytosine-rich, with 32±4 mol% C, 23±5 mol% G, 21±0 mol% A and 23±1 mol% T. This molecule contained five ORFs with coding capacities for proteins greater than 5 kDa, located between an untranslated leader sequence of 278 nt and a 3’ non-coding sequence of 185 nt. However, only ORFp17 and ORFp70, which partially overlap and begin at the first two AUG codons, are likely to be cistrons that are expressed during the replication of DpTV. This organization is very similar to those of HaSV RNA2 and NoV RNA2 (Hanzlik et al., 1995). ORFp17 begins with the first AUG and encodes a predicted 158 aa, 16 801 Da protein. The context of ORFp17 (CUCAUGA) for translation initiation matched four of seven nucleotides of the consensus sequence (A/G-CCAUGG) for initiation of eukaryotic protein translation (Kozak, 1986). ORFp70 begins with the second AUG and encodes a protein of 643 aa, with a molecular mass of 69 744 Da. The context of ORFp70 (AGAAUGG) matched five of seven nucleotides in the Kozak consensus sequence. The nucleotide sequence of DpTV RNA2 was 74 and 63 % identical to those of NoV and HaSV, respectively, and is longer than both (by 12 and 42 nt, respectively).

Both DpTV RNAs contained a 3’-terminal CCA box that might serve as an RNA initiation signal for the viral RNA-dependent RNA polymerase (Yoshinari et al., 2000). Analysis of the nucleotide sequences revealed no significant ORFs in the complementary-sense strands of DpTV RNA, indicating that DpTV is a positive-stranded virus.

The 180 kDa protein
The 1649 aa protein (p180) showed a great degree of similarity to the replicases encoded by HaSV and NoV. Like these two viruses, the replication protein of DpTV contained the three major functional domains identified in the replicases of positive-stranded RNA viruses (Koonin et al., 1992; Koonin & Dolja, 1993). These are, in order from the N terminus, the putative methyltransferase domain, the nucleotide-binding or helicase domain and the polymerase domain. Alignment of the DpTV replicase with those of NoV and HaSV revealed a few highly conserved residues within the motifs that define these domains (Fig. 4).

The N-terminal region (aa 50–216) of the DpTV replicase corresponded clearly to the putative methyltransferase

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**Fig. 4.** Comparison of deduced amino acid sequences of DpTV replicase proteins with conserved motifs from HaSV and NoV. Shown are alignments of methyltransferase (a), helicase (b) and polymerase (c) domains. Roman numerals indicate conserved motifs designated by Koonin et al. (1992). Asterisks indicate amino acid residues within these motifs that are highly conserved among a range of virus families in the alpha-like superfamily (Koonin et al., 1992; Gordon et al., 1995). Numbers on the left indicate the starting amino acid positions of the aligned sequences. Residues identical in at least two of the viruses are shown on a black background.
domain (Fig. 4a). All six of the conserved motifs identified by Candresse et al. (1990) and Rozanov et al. (1992) in plant viruses and alphaviruses, respectively, were present in this region. The sequence of the methyltransferase domain of DpTV shared 67 % identity with that of HaSV and 38 % with that of N\textit{b}\textit{V}.

Amino acids 594–840 of the DpTV replicate specified the putative nucleotide-binding or helicase domain (Fig. 4b). All seven conserved motifs of the superfamily I helicase domain (Koonin & Dolja, 1993) were detected in the region, which Candresse et al. (1990) concluded to be the alpha-like consensus elements. Alignment of the amino acid sequences of the helicase domains showed that DpTV and HaSV were distantly related, with 63 % identity. In contrast, N\textit{b}\textit{V} is related very closely to HaSV, with 88 % identity in this domain (Gordon et al., 1999).

The DpTV RNA-dependent RNA polymerase (aa 1042–1270) contained all eight conserved motifs that were identified by Poch et al. (1989), Candresse et al. (1990) and Koonin & Dolja (1993) as common to the RNA-dependent RNA polymerase of replicates of positive-stranded RNA viruses (Fig. 4c). The three motifs IV, V and VI showed completely unequivocal conservation. They were located at aa 1123–1128 (DX\textsubscript{4}D), 1181–1190 (SG\textsubscript{X}\textsubscript{1}TX\textsubscript{N}) and 1212–1214 (GDD). The sequence of the RNA-dependent RNA polymerase domain of DpTV shared 76 % identity with that of HaSV and 36 % with that of N\textit{b}\textit{V}.

The DpTV replicate has a long (379 aa) C-terminal extension beyond the end of the polymerase domain. The corresponding regions of N\textit{b}\textit{V} and HaSV are 757 and 481 aa long, respectively. This C-terminal sequence of the DpTV replicate is 15:1 and 38:2 % identical to those of N\textit{b}\textit{V} and HaSV, respectively, but has no significant homology to replicates of other virus families. The presence of this long C-terminal domain in both genera of the tetraviruses suggests that it has some function (albeit unknown), according to Gordon et al. (1999).

The 17 kDa protein

Analysis of p17 by using BLAST revealed that the amino acid sequence had no putative conserved domains, but had a high degree of overall identity (44 %) to the p17 of HaSV, which has 157 aa. Surprisingly, identity between the N and C termini of these two proteins was as high as 65:5 and 53:6 %, respectively. Like the p17 of HaSV, the p17 of DpTV also has a high content (48:1 %) of PEST amino acids, with 15:8 % Pro, 3:8 % Glu, 15:8 % Ser and 12:7 % Thr. The function of this protein remains unknown, but Hanzlik et al. (1995) postulated that it might function as a regulatory and movement protein.

The 70 kDa protein

The putative amino acid sequence of p70 had significant similarity to the known capsid protein precursors of other tetraviruses, suggesting that p70 might be the capsid precursor of DpTV. Multiple sequence alignment of the DpTV capsid precursor sequence with those of five other tetraviruses revealed that DpTV was related more closely to N\textit{o}\textit{V} than to HaSV, PrV, N\textit{b}\textit{V} or TaV. The DpTV capsid precursor shared 86 % identity with that of N\textit{o}\textit{V}, 66 % with that of HaSV, 30 % with that of PrV, 23 % with that of N\textit{b}\textit{V} and 21 % with that of TaV. Interestingly, N\textit{b}\textit{V} and N\textit{o}\textit{V} infect the same host insects, but N\textit{o}\textit{V} is related most closely to DpTV, whereas N\textit{b}\textit{V} is almost the most distantly related of all the sequenced tetraviruses.

Eight regions of \(\beta\)-sheets were identified in DpTV that align with those found in the crystal structure of N\textit{o}\textit{V}. The presence of these regions suggests that the capsid of DpTV exhibits the jelly-roll structure that is expected of an icosahedral virus particle.

A phylogenetic tree was generated with CLUSTAL \textit{W} based on the conserved sequence predicted for the \(\beta\)-sheets of the capsid proteins (Fig. 5). The outgroup, \textit{Black beetle virus} (BBV), was placed on one branch, whilst the other branch contained all the tetraviruses. This branch was further divided into two clades. DpTV was related most closely to N\textit{o}\textit{V}, followed by HaSV. Although PrV was a member of the N\textit{b}\textit{V}-like viruses, it was related more closely to the N\textit{o}\textit{V}-like viruses. These four viruses seemed to belong to one clade, whereas the other two N\textit{b}\textit{V}-like viruses were grouped together.

Secondary-structural features of DpTV RNA

Both 3′-terminal sequences of the DpTV RNAs (131 nt of RNA1 and 171 nt of RNA2) can be folded into tRNA-like secondary structures with a valine anticodon, similar to those structures previously identified on the HaSV and N\textit{b}\textit{V} RNAs (Fig. 6). These structures differ from the known plant virus tRNA-like structure in that it forms a pseudoknot in the aminoacyl stem, but with the clover-leaf folding of canonical tRNA. Secondary-structure analysis of the 3′ terminal sequences using the Mfold program detected helices and stem–loops II–VI. These were
confirmed by analysis with the STAR program (Abrahams et al., 1990; Gultyaev, 1991) and by eye, which also resulted in the detection of stem I. The RNA1 and RNA2 3' terminal sequences are about 65% identical, but the sequence differences between these two RNAs do not disrupt the tRNA-like structure.

The sizes of the 5' untranslated leading sequences of the DpTV RNAs are similar to those of the HaSV RNAs. There is no hairpin structure, which contained the conserved hexamer GGUAAA in the loop, identified by Gordon et al. (1995), on both genomic RNAs of HaSV.

DISCUSSION

The characteristics that we observed for DpTV most closely match those of the family Tetraviridae. The buoyant density of DpTV virions in CsCl, the size of the icosahedral particle, the molecular masses of the capsid protein components and the presence of two single-stranded RNA molecules are all recognized features of the Tetraviridae (Hanzlik & Gordon, 1997). The genomic organization and the identities of the capsid protein precursors and the replicases support the inclusion of this virus in the genus Omegatetravirus of the family Tetraviridae.

Cap structures have been observed on the 5' termini of the HaSV genomic RNAs (Gordon et al., 1995; Hanzlik et al., 1995) and are likely to be observed in other tetraviruses, according to Hanzlik & Gordon (1997). To determine whether the 5' termini of DpTV RNAs are capped, we synthesized the first cDNAs for 5' RACE in the presence of different reverse transcriptases by using primers designed according to the known sequences. For each strand of DpTV RNA, we chose 20 5' RACE clones to sequence, none of which was confirmed to have a guanine residue at the 5' terminus. We therefore postulate that neither RNA1 nor RNA2 has a 5' cap, but enzymological analysis will be required to confirm this.

When we used 3' RACE to determine the 3' terminal sequences of the DpTV RNAs, we were unsuccessful in our attempts to prime cDNA synthesis with oligo(dT) on total RNA extracted from virions. This indicates that the RNAs lack extensive regions of polyadenylation, although we could not rule out the possibility that the 3' ends contain an oligo(A) tract that is short enough that the RNAs could not be distinguished from in vitro-added poly(A) during 3' RACE. Accordingly, when we used 3' RACE to complete the 3' terminal sequences, in addition to the method described above, we also used a method described by Lambden et al. (1992). In brief, the 3' terminus of each strand was treated with T4 RNA ligase in the presence of an adapter (5'-PO4-CCCGATCCGTCGACGAATTCTTT-NH2) and reverse-transcribed by using the complementary primer (5'-AAAGAATTCGTCGACGGATCCGGG-3'), then amplified with the complementary primer and the special primer based on the known sequences. We concluded that both RNA1 and RNA2 of DpTV contain a 3' terminal CCA box.

It is known that the tetraviruses have two different strategies of capsid-protein expression. In NpV, NoV and HaSV, the large and small capsid proteins are generated by cleavage of the capsid protein precursors at a single Asn–Phe or Asn–Gly site, and the N termini of the large capsid proteins are blocked (Agrawal & Johnson, 1992; Hanzlik et al., 1995; Gordon et al., 1999). In contrast, in TaV and PrV, the precursor is cleaved twice to produce an unidentified non-structural protein in addition to the large and small capsid proteins (Pringle et al., 1999, 2003). Our examination of DpTV capsid proteins with SDS-PAGE revealed two bands with molecular masses of about 62 and 7 kDa (Fig. 2a, b), so the molecular mass of the capsid protein precursor would...
be expected to be about 69 kDa. This is almost equal to the coding capacity (69744 Da) of the capsid gene of DpTV. According to the homology between the DpTV capsid protein precursor and those of NoV and HaSV, we postulate that the capsid protein precursor of DpTV is cleaved once at an Asn–Phe site located after residue 570, producing two proteins with molecular masses of 62 126 and 7634 Da. These sizes are highly similar to the molecular masses determined by SDS-PAGE for the DpTV capsid proteins.

The tetravirus 3′ tRNA-like structures are the only ones that have been identified in animal viruses (Hanzlik & Gordon, 1997). It is not known whether the DpTV structures can be aminoc酰lated like those of HaSV. Gordon et al. (1999) showed that the tRNA-like structures are likely to be involved in RNA replication in vivo. It has also been proposed that these structures protect the RNA from exonucleolytic degradation.

Tetraviruses are often found in the presence of other viruses. The first reports on NjV presented it as one of five viruses that were found in a batch of diseased insects (Juckes, 1970) and HaSV has also been found in the presence of a reo-like virus, a picorna-like virus and an as-yet-unclassified small virus (Hanzlik & Gordon, 1997). When we isolated DpTV, we also found it to co-exist with two other viruses, a cytoplasmic polyhedrosis virus and a small virus whose diameter is about 20 nm (the subject of further study). Whether DpTV and the other two viruses are found in the same animal, or in separate individuals within a batch, is unclear. Experiments on D. punctatus pathology are currently under way and might provide definitive answers to this question.

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