Sequence of the non-structural protein gene encoded by RNA1 of striped jack nervous necrosis virus

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Striped jack nervous necrosis virus (SJNNV), the causative agent of viral nervous necrosis in marine fish, is a member of the family Nodaviridae whose genome consists of two positive-sense RNA molecules encapsidated in a single virion. In this study, the nucleotide sequence of SJNNV RNA1 was determined. The SJNNV RNA1 was 3081 bases long and contained a single ORF encoding 983 aa of approximately 110 kDa. The sequence identities between RNA1 of SJNNV and RNA1 of insect nodaviruses were 28% at the nucleotide and amino acid levels, although the conserved motifs for the RNA-dependent RNA polymerase were located at almost the same positions in the amino acid sequences. The present study, together with our previous work on SJNNV RNA2, suggests that a new genus, Piscinodavirus, should be created in the family Nodaviridae.

Fish nodaviruses, members of the family Nodaviridae, are the causative agents of viral nervous necrosis (VNN) or fish encephalitis, producing high mortality in hatchery-reared larvae and juveniles of marine fishes in Japan, South-East Asia, Australia and Europe (Mori et al., 1992; Compes et al., 1994; Munday & Nakai, 1997). VNN has been recorded in 22 species of marine fishes (Muroga et al., 1998). Striped jack nervous necrosis virus (SJNNV), the causative agent of VNN in larvae of striped jack (Pseudocaranx dentex), is the best-studied member of the fish nodaviruses. SJNNV consists of a single type of coat protein and two single-stranded, positive-sense RNAs, RNA1 and RNA2, which lack a poly(A) structure. SJNNV RNA1 encodes a non-structural protein of approximately 100 kDa, whereas RNA2 encodes a coat protein of approximately 42 kDa (Mori et al., 1992). PCR primers were designed based on the nucleotide sequence of SJNNV RNA2 (Nishizawa et al., 1994). In the RNA2 of fish nodaviruses, a highly conserved region and a variable region have been described and the fish nodaviruses have been classified into four different genotypes by molecular phylogenetic analyses based on nucleotide sequences of the variable region (Nishizawa et al., 1995, 1997).

Currently, the family Nodaviridae is made up of one genus, Nodavirus, which contains insect nodaviruses such as Nodamura virus (NOV), black beetle virus (BBV), Flock House virus (FHV) and Boolarra virus (Hendry et al., 1995). The genomic RNA2 of insect nodaviruses is a coat protein gene and encodes a protein x, the precursor of virion proteins β and γ, whereas the genomic RNA1 encodes two non-structural proteins, protein A (110 kDa; the RNA-dependent RNA polymerase (RDRP)] and protein B (11 kDa; the translation product of subgenomic RNA (RNA3) which is derived from RNA1 during replication) (Gallagher et al., 1983; Dasmahapatra et al., 1985; Kaesberg et al., 1990; Ball, 1995). Fish nodaviruses are clearly distinguished from insect nodaviruses based on a comparative study of the coat protein gene. Thus, a new genus has been proposed in the family Nodaviridae to include the fish nodaviruses (Nishizawa et al., 1995).

Little is known about the primary structure of fish nodavirus RNA1 and the non-structural protein encoded by it. In this study, SJNNV RNA1 was cloned and sequenced. Analysis showed that the non-structural protein of SJNNV is RDRP and again emphasizes that fish nodaviruses can be clearly distinguished from insect nodaviruses.

Striped jack larvae infected with SJNNV were collected during an outbreak of VNN at a hatchery in Japan and stored at −20 °C. Whole bodies of infected larvae were homogenized with 9 volumes of Dulbecco’s PBS (D-PBS) and centrifuged (10000 g, 10 min, 4 °C). The supernatant was filtered through a membrane (0.45 μm; Advantec) and centrifuged (150000 g, 40 min, 4 °C). The pellet was resuspended in D-PBS and centrifuged (100000 g, 60 min, 4 °C) through a discontinuous gradient composed of 20, 35 and 50% (w/v) sucrose in D-PBS. The virus was collected from the interface between 35 and 50% (w/v) sucrose and pelleted by centrifugation (150000 g, 40 min, 4 °C). The virus pellet was resuspended in CsCl (ρ = 1.32) and centrifuged (100000 g, 12 h, 4 °C). The virus was collected, diluted and then pelleted by centrifugation (150000 g, 40 min, 4 °C). The pelleted viral...
Fig. 1. Schematic illustration of the physical map of the SJNNV RNA1, cloned plasmids for nucleotide sequence analysis and ORFs found in the determined nucleotide sequence. Thick arrows indicate the ORFs for protein A and putative protein B. Thin arrows show relative locations of cDNAs against the RNA1 and directions of cDNAs in the multi-cloning site of the plasmid (from T3 to T7 promoter sites) of cDNAs in the plasmids.

particles were resuspended in TE (10 mM Tris–HCl, pH 8.0, 1 mM EDTA). After phenol and chloroform extraction, cDNAs were synthesized using a cDNA synthesis kit (Pharmacia). After addition of EcoRI adapters, the cDNAs were ligated into the plasmid vector, pBluescript KS(−) (Stratagene) and used to transform Escherichia coli DH5α (Toyobo). The recombinant plasmids were labelled with a digoxigenin labelling kit (Boehringer Mannheim) and hybridized with SJNNV RNAs to select recombinant plasmids with a cDNA specific for viral RNA1. Nucleotide sequences were determined with a dye terminator cycle sequencing kit (ABI) and analysed with the autosequencer A373-36 (ABI). Sequences were assembled and analysed with the computer program MacDNASISpro (Hitachi Software Engineering).

The schematic illustrations of the physical map of SJNNV RNA1 and recombinant plasmids used for the sequence analyses are shown in Fig. 1. A total of 13 plasmids was needed to perform the sequence analyses of SJNNV RNA1. The nucleotide and deduced amino acid sequences of SJNNV RNA1 are shown in Fig. 2. The nucleotide sequence was 3081 bases in length containing an ORF at nt 65–3016. Thus, there are 65 bases of 5′-non-coding region and 65 bases of 3′-non-coding region. The molecular mass of SJNNV RNA1 calculated from the determined sequence was 1,01 × 10^6 Da, which corresponds to the estimated molecular mass of RNA1 obtained by PAGE (Mori et al., 1992). A polypeptide of 983 aa encoded by the ORF of SJNNV RNA1 has a molecular mass of approximately 111 kDa, which agrees with the size of the polypeptide translated from SJNNV RNA1 (Mori et al., 1992).

The RNA1 of insect nodaviruses are approximately 3100 bases in length and contain an ORF encoding a protein of 998 aa with a calculated molecular mass of 112 kDa (Dasmahapatra et al., 1985; Ball, 1995). The length of SJNNV RNA1 and its encoded polypeptide were slightly shorter. The sequence identities of RNA1 between SJNNV and the insect nodaviruses BBV and FHV were 28–3% at the nucleotide level and 27–6% at the amino acid level, whereas the values between BBV and FHV were more than 99–0% at both nucleotide and amino acid levels. A conserved sequence was not found in the nucleotide and amino acid sequences between RNA1 of SJNNV and insect nodaviruses by matrix plots analysis (window size, 10; minimum score, 80%) (data not shown).

In the RNA1 sequences of the insect nodaviruses BBV and FHV, there are 38 bases of 5′-non-coding region (of which 55% is A) and 74 bases of 3′-non-coding region. There is a stem–loop structure in the first 19 bases of the 5′-non-coding region and two stem–loop structures at the 3′ terminus (Dasmahapatra et al., 1985; Kaesberg et al., 1990). The 64 bases of the 5′-non-coding region in SJNNV RNA1 contained 28% A. Stem–loop structures corresponding to those of insect nodaviruses were not detected in the non-coding regions. These results indicate that SJNNV and insect nodaviruses are related but distinguishable from each other. We believe that a new genus, Piscinodavirus, should be created in the family

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Nucleotide sequence of SJNNV RNA1

Fig. 2. Determined nucleotide sequence of SJNNV RNA1 and deduced amino acid sequences encoded by the RNA1. A large polypeptide [protein A (RDRP)] and a small one (putative protein B) are shown. Underlined amino acids show the conserved motifs of RDRP.

Nodaviridae, as has been previously proposed based on analysis of RNA2 (Nishizawa et al., 1995).

The sequence motif YXDD is highly conserved amongst RNA polymerase, DNA polymerase and reverse transcriptase. In positive-strand RNA viruses, GDD is the consensus sequence (Delarue et al., 1990; Poch et al., 1989). In fact, the eight conserved motifs, including the GDD motif, are identified from amino acid sequence alignment of viral RDRPs, and six of them in the RDRP of SJNNV.
them are found in the protein A of insect nodavirus: the acidic motif at aa 582–587 (motif 1); the SG.T motif at aa 649–654 (motif 2); the GDD motif at aa 691–693 (motif 3); the basic motif at aa 716 (motif 4); motif 7 is a basic sequence preceded by an aromatic residue at aa 796; and motif 8 is a basic residue preceded by a basic sequence at aa 819 (Bruenn, 1991). As described above, the non-structural protein of SJNNV and the protein A of insect nodaviruses showed only 27.5% amino acid sequence identity. However, all of the six conserved motifs observed in the protein A of insect nodaviruses were found at aa 585–590 (motif 1), aa 646–651 (motif 2), aa 680–688 (motif 3), aa 712 (motif 4), aa 788 (motif 7) and aa 808 (motif 8) of the non-structural protein of SJNNV. Interestingly, each conserved motif was located at almost the same position in the SJNNV and insect nodavirus proteins. It seems highly likely that the non-structural protein encoded by SJNNV RNA1 is RDRP.

The RNA1 of insect nodaviruses encodes two non-structural proteins, protein A (RDRP) and protein B (whose function is unknown). Protein B, with a molecular mass of 11 kDa, is the translation product of subgenomic RNA3, which is derived from the 3′ end of RNA1 during virus replication. The ORF for protein B is found at nt 2737–3057 in RNA1 (Gallagher et al., 1983; Guarino et al., 1984; Ball, 1995). No report has been made concerning a putative protein B of fish nodaviruses, although an RNA3, with approximately 400 bases, was detected in cells of sea bass larva (Dicentrarchus labrax) infected with the fish nodavirus Dicentrarchus labrax encephalitis virus (Delsert et al., 1997). Although the existence of RNA3 has not yet been confirmed for SJNNV, a potential ORF for protein B is found at nt 2742–2969 in the determined nucleotide sequence of SJNNV RNA1 (Fig. 2). The calculated molecular mass from the deduced amino acid sequence was approximately 8.4 kDa, which is a little smaller than that of the insect nodavirus protein B. However, further studies will be needed to show whether the peptide encoded by this ORF of SJNNV RNA1 corresponds to the protein B of insect nodaviruses. Mapping of a neutralizing epitope on the coat protein of SJNNV is discussed in the accompanying paper by Nishizawa et al. (1999).

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