Characterization of a new picorna-like virus, himetobi P virus, in planthoppers

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Picorna-like virus particles, 29 nm in diameter, were purified from apparently healthy Laodelphax striatellus Fallen. The virus particles had a buoyant density of 1.352 g/ml in CsCl and a sedimentation coefficient of 161 s. The virus capsid proteins consisted of three major polypeptides of Mr 36 500, 33 000 and 28 000, and three minor polypeptides. The virus contained a major ssRNA of Mr 2.8 x 10^6 and was also frequently associated with a minor dsRNA of Mr 4 x 10^6. The 3' end of the ssRNA had a poly(A) tract of about 60 adenine residues. The virus has been provisionally named himetobi P virus.

The small brown planthopper, Laodelphax striatellus (Fallen) is an important vector of virus diseases of graminaceous plants. It is the most important active vector of rice stripe tenuivirus (RSV) diseases in the rice-growing areas of Japan (Toriyama, 1983; Conti, 1985; Kisimoto & Yamada, 1986).

An isometric virus was detected while attempting to purify RSV from its planthopper vector, L. striatellus. The virus was detected, using an ELISA, in three planthopper species from Japan. High incidences of infection also occurred in some laboratory cultures of L. striatellus, Sogatella furcifera (Horv.) and Nilaparvata lugens (Stal.) (Toriyama et al., 1991; Guy et al., 1992). In the field, the virus was detected at a low incidence in these planthopper species. Here we report the characteristics of the virus from L. striatellus.

Small brown plant hoppers were reared continuously on rice seedlings under laboratory conditions (courtesy of Dr T. Matsui of Agriculture Research Center, Tsukuba, Japan). Insects were harvested at the late adult growth stage and were stored frozen at -20 °C until needed. Virus purification followed, with slight modification, the procedure for RSV (Toriyama & Watanabe, 1989). One to 8 g of frozen planthoppers was macerated with 100 ml of extraction solution (100 mM-Na2HPO4, 10 mM-sodium diethyldithiocarbamate, ascorbic acid added to give pH 7.2) and clarified using Difron S-3 (40 ml, 28% final volume; Daikin). After a cycle of 4200 g and 123 000 g centrifugation, the pellets were resuspended in 10 mM-potassium phosphate buffer pH 7.5. The preparation was centrifuged in 10 to 40% linear sucrose gradients. Contaminating host components were completely separated and removed by rate-zonal centrifugation in 1-4 M-Cs2SO4 for 16 h at 100 000 g at 10 °C. The buoyant density of the virus particles was determined as described previously (Toriyama et al., 1983). The virus preparations were centrifuged on CsCl gradients for 70 h at 90 000 g. The sedimentation coefficient of the virus (A260, 0.4 to 0.8) was measured with an Analytical Ultracentrifuge System (Absorption Scanner ABS 8, Hitachi). The Mr values of viral capsid proteins were determined in 10% SDS-polyacrylamide gels (Laemmli, 1970) and 8 to 16% linear gradient SDS-polyacrylamide gels. Virus nucleic acid was characterized as described previously (Toriyama & Watanabe, 1989) and the 3'-terminal sequence of the viral RNA was determined by two-dimensional shift analysis and sequencing with an RNase sequencing kit (Pharmacia) (Wengler et al., 1982; Takahashi et al., 1990).

Double diffusion tests in 1% agar were done using the virus from L. striatellus and antisera to Rhopalosiphum padi virus (courtesy of Dr C. D’Arcy), sacbrood picornavirus (Dr D. Anderson), infectious flacherie virus (Dr M. Nagata) and flock house nodavirus (Dr P. Scotti). There were no serological reactions between these antisera and the virus from L. striatellus, but an antiserum produced against this virus had a reciprocal homologous titre of 4096.

Viral preparations formed a sharp band on Cs2SO4 gradients which contained homogeneous isometric particles approximately 29 nm (28 to 30 nm) in diameter (Fig. 1). The virus yield was 20 to 50 µg/g of plant hoppers. The buoyant density of the virus particles...
was 1.352 ± 0.02 g/ml and the sedimentation coefficient was estimated to be 161 s (160 to 162 s). As shown in Fig. 2 (a), the three major capsid proteins, $M_r$ 36 500, $M_r$ 33 000 and $M_r$ 28 000, had similar staining density ratios. Another three minor components with $M_r$ s of 41 500, 31 500 and 31 000 were always associated with the viral preparations. In some insect picornaviruses, a minor protein component of $M_r$ 36 500, which is assumed to correspond to VP0 of mammalian picornaviruses, is present (Moore et al., 1985). However, the origin and function of the other two minor components are unclear.

Electrophoresis of nucleic acid extracts in 1% agarose gels revealed two components, although the amount of the slower migrating component varied in different viral preparations (Fig. 2b). DNase treatment (DNase 1, RNase-free; Boehringer Mannheim) did not digest either component, whereas λ phage DNA (HindIII digest) was completely digested under these experimental conditions. The faster migrating major component was digested by RNase A under high and low salt (0.3 M- and 0.03 M-NaCl) conditions whereas the slower migrating minor component was resistant to RNase A in 0.3 M-NaCl. This indicated that the major component was ssRNA ($M_r$ 2.8 × 10$^6$) and that the minor component was dsRNA ($M_r$ 4 × 10$^6$). Most of the 3' $^{32}$P-labelled viral RNA was bound to an oligo(dT)-cellulose column (Type 7, Pharmacia) and eluted with the appropriate elution buffer, showing that the RNA contained a poly(A) tract (data not shown). This was confirmed by the two-dimensional shift analysis. The terminal nucleotide of ssRNA was adenine (A) and the wandering spots in Fig. 3(a) resolved an ssRNA 3'-terminal tract of 14 A residues. A tract of approximately 60 A residues was confirmed by RNA sequencing.

Fig. 1. Virus particles purified from $L$. striatellus and stained with 2% phosphotungstate. Bar marker represents 100 nm.

Fig. 2. (a) Analysis of viral proteins of $L$. striatellus, subjected to electrophoresis through an 8 to 16% linear SDS-polyacrylamide gel (Funakoshi Ltd) and stained with Coomassie blue. Lane 1, marker proteins (Pharmacia), from upper band: phosphorylase b (94K), albumin (67K), ovalbumin (43K), carbonic anhydrase (30K), trypsin inhibitor (21.1K) and lactalbumin (14.4K). Lane 2, capsid proteins of the virus. Arrows show the positions of the minor proteins. (b) Nucleic acid extracted from the virus, subjected to electrophoresis through a 1% agarose gel and stained with ethidium bromide. Lane 1 (from top), minor dsRNA and major ssRNA. Lanes 2 and 3, ss- and dsRNAs for $M_r$ markers. Position and $M_r$ ($\times 10^{-6}$) of RNA markers are shown on the right (ssRNAs) and left (dsRNAs); these were ssRNAs of RSV (3, 3.6, 1.4 and 0.9), tobacco mosaic virus (OM strain) (2) and dsRNAs of RSV (5, 2.5, 1.5 and 0.9) (Toriyama & Watanabe, 1989).

Fig. 3. Autoradiograms of wandering spots of (a) ss- and (b) dsRNAs of the virus from $L$. striatellus. The 3' end of the RNA was labelled with 5' [32P]PCP using T4 RNA ligase and subjected to electrophoresis through a 1% agarose gel. Each band of ss- and dsRNA was cut out, RNA was electrophoretically eluted and this was followed by two-dimensional shift analysis (Takahashi et al., 1990). The line of wandering spots on the right side of (b) was more visible after longer exposure.
(RNase kit, Pharmacia), suggesting a poly(A) tract of variable length. The 3' end of the dsRNA was also labelled and a similar poly(A) tract was determined (Fig. 3b). An additional line of poorly labelled wandering spots was observed in the dsRNA after long exposure (Fig. 3b, right side). A similar wandering spot pattern has been reported in the replicative form of Sindbis virus RNA with a poly(A) tract (Wengler et al., 1982). The terminal nucleotides of the dsRNA were A (major component) and uridine (U) (minor component). The terminal nucleotide U may correspond to the poorly labelled 3' end sequence which is complementary to the 5' end of the RNA molecule with the 3' end poly(A) tract of dsRNA (Fig. 3b).

As the dsRNA is found in highly purified viral preparations, it seems unlikely to be a replicative form RNA. It is similar to the dsRNA that was found in RSV ss- and dsRNAs (Toriyama & Watanabe, 1989).

The characteristics of the viral capsid proteins and the ssRNA, and physical properties of the viral particles suggest that the virus of small brown planthoppers may belong to the Picornaviridae (Francki et al., 1991; Moore et al., 1985, 1987; Williamson et al., 1988). No serological relationships were found between the picorna-like virus of L. striatellus, the aphid virus of R. padi, infectious flacherie virus or sacbrood virus. Cricket paralysis and Drosophila C picornaviruses are serologically related to a number of other insect picornaviruses. Unfortunately antiserum to these viruses were unavailable. The capsid proteins of Drosophila C virus and cricket paralysis virus (Victoria isolate) include a small protein with an Mr of approximately 8000 (Jousset et al., 1977; Scotti et al., 1981), which was not observed in the picorna-like virus of planthoppers; however the presence of a minor dsRNA component seems to be a characteristic of this virus. We named the new picorna-like virus of planthoppers himetobi P virus after the Japanese name for the small brown planthopper.

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References


