Immunological characterization of rice tungro spherical virus coat proteins and differentiation of isolates from the Philippines and India

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Rice tungro spherical virus (RTSV) has an RNA genome of more than 12 kb with various features which classify it as a plant picornavirus. The capsid comprises three coat protein (CP) species, CP1, CP2 and CP3, with predicted molecular masses of 22.5, 22.0 and 33 kDa, respectively, which are cleaved from a polyprotein. In order to obtain information on the properties of these proteins, each was expressed in E. coli, purified as a fusion to the maltose-binding protein and used for raising a polyclonal antiserum. CP1, CP2 and CP3 with the expected molecular masses were detected specifically in virus preparations. CP3 is probably the major antigenic determinant on the surface of RTSV particles, as was shown by ELISA, Western blotting and immunogold electron microscopy using antisera obtained against whole virus particles and to each CP separately. In some cases, especially in crude extracts, CP3 antiserum detected several other proteins (40–42 kDa), which could be products of CP3 post-translational modification. No serological differences were detected between the three CPs from isolates from the Philippines, Thailand, Malaysia and India. The CP3-related 40–42 kDa proteins of the Indian RTSV isolate have a slightly higher electrophoretic mobility (42–44 kDa) and a different response to cellulolytic enzyme preparations, which allows them to be differentiated from south-east Asian isolates.

Introduction

Rice tungro disease is caused by a combination of rice tungro spherical virus (RTSV), which provides the leafhopper transmission characters, and rice tungro bacilliform virus (RTBV), which contributes most of the symptoms. RTSV has been classified as a member of the recently established family Sequiviridae (Murphy et al., 1995). It has a polyadenylated RNA genome which is more than 12 kb in length. The nucleotide sequence of the full-length RNA of a Philippine isolate of RTSV (Shen et al., 1993) and the partial nucleotide sequences of isolates from other south-east Asian countries (Malaysia, Thailand, India and Bangladesh; Zhang et al., 1993 b) have been determined, revealing some variation. The genome organization of RTSV (Fig. 1), predicted from the RNA sequence (Shen et al., 1993), appeared to be similar to some representatives of the animal picornavirus group. RTSV has one large open reading frame (ORF), encoding a polyprotein which is thought to be cleaved by virus- and/or cell-encoded proteases. The genome also contains two other small ORFs, which are thought to be expressed from subgenomic mRNAs (Shen et al., 1993). Morphological, biochemical and phytopathological characteristics of RTSV are similar to those of maize chlorotic dwarf virus, but the amino acid sequence of the polyprotein has several significant similarities to those of members of the comovirus and nepovirus groups. Experimental data supporting RTSV functional organization have not yet been published.

The RTSV capsid comprises three coat proteins, CP1, CP2 and CP3, with predicted molecular masses of 22.5, 22.0 and 33 kDa, respectively, which are processed from the polyprotein at amino acid positions 645–852, 853–1055 and 1056–about 1350 within viral RNA (see Fig. 1). These positions were predicted from N-terminal sequencing of the
CPs (Shen et al., 1993; Zhang et al., 1993a); the precise site of the C terminus of CP3 has not yet been determined.

Previous immunological studies of RTSV using polyclonal antisera obtained against whole virus particles did not elucidate properties of individual CPs in vivo because of a relatively low titre and specificity. Although virus detection systems developed using such antisera proved effective (Bajet et al., 1985; Hibino & Cabauatan, 1985), it was considered necessary to raise antisera specific to each virus protein if questions relating to virus structural and functional organization and variation were to be addressed.

Based on symptomatology, distinct strains of tungro from the Philippines (Rivera & Ou, 1967) and India (Anjaneyulu & John, 1972; Mishra et al., 1976) and several other countries (Hibino, 1987) have been previously reported. However, recently it has been shown that rice cultivars react differentially to RTSV and/or RTBV and express variable symptoms depending on infection with either one or both of these viruses (Hibino et al., 1987; Dahal et al., 1990). Therefore, it is apparent that differentiation of tungro strains cannot be based simply on the symptomatology. Sequence analysis and cross-hybridization experiments have shown that there are basically two groups of RTBV isolates: one from the Indian subcontinent and the other from the Philippines and Malaysia (Fan et al., 1996). There have not been any serological studies on variation of tungro viruses.

The aims of the present study were to raise polyclonal antiserum against each RTSV CP which was expressed separately in E. coli and to use these antisera for immunological characterization of different preparations and isolates of the virus.

**Methods**

**Host plants.** Isolates of tungro from the Philippines, India and Malaysia have been described by Dahal et al. (1992); the Thai isolate was from the Department of Agriculture, Bangkhen, Thailand. These isolates were maintained either as RTSV + RTBV or as RTSV alone in rice cv. TN1 under greenhouse conditions. The virus was transmitted by feeding the virus-carrying *Nephotettix virescens* on approximately 12-day-old rice seedlings in a cage for 24 h. After 2-3 weeks, plants were used for virus preparation and immunological analysis.

**Virus preparation.** Highly purified RTSV (Philippine isolate), prepared using driselase (Cabauatan & Hibino, 1988), was kindly provided by H. Koganazawa (IRRI, Philippines). Semi-purified virus preparations were obtained using the method involving the cellulosytic enzyme Celluclast (Novo Industri) described by Jones et al. (1991). In some cases, different cellulosytic enzymes were used: cellulases, SP249 batch KRN0013, SP348 and Steepzyme batch KRN2002 (from Novo Industri) at 5% in 0.1 m-citrate buffer, pH 7.0, and cellulase Onozuka R5 (Yakult Honsha) and Pectolyase Y-23 (Seishin Pharmaceutical) at 0.25 mg/ml in the same buffer. Alternatively, cellulase treatment was omitted from the virus purification or was performed after purification.

For crude protein preparations, sap from 0.1-0.5 g rice leaves was extracted with PBS using a sap press (Dalichow). The extracts were clarified by centrifugation at 12000 g for 5 min, filtered through a 0.2 μm membrane filter (Schleicher & Schuell) and kept frozen at -20 °C. Extracts were concentrated using Ultrafree-MC (Millipore) microcentrifuge filters.

**Virus RNA extraction.** The virus particles were disrupted in 0.4% SDS at 65 °C for 5 min. The RNA was extracted with phenol-chloroform, precipitated with 2 m-LiCl at 4 °C overnight, recovered by centrifugation at 12000 g, washed with 70% ethanol and resuspended in TE buffer (10 mM-Tris-HCl, 1 mM-EDTA, pH 8.0).

**RT–PCR of RTSV CPs and cloning in pMAL-c2.** First-strand cDNA was synthesized using a primer (primer 10; Fig. 1) which is complementary to RTSV RNA downstream of the presumed C terminus of CP3. The individual CP regions and part of the RTSV cDNA downstream of CP3 (DS) (see Fig. 1) were then prepared from this cDNA by PCR (94 °C, 1 min; 55 °C, 2 min; 72 °C, 3 min; 30 cycles) with the primers listed in Fig. 1.

Individual PCR products were purified on an agarose gel, cut with the appropriate restriction enzyme and ligated into the digested pMAL-c2 (New England Biolabs) E. coli expression vector, which directed the synthesis of foreign polypeptides as C-terminal fusions of the 42 kDa maltE gene product, maltose-binding protein (MBP) (Guan et al., 1987; Maina et al., 1988). The DS fragment was also inserted in-frame with lacZ into pUC19 and expressed to assess the specificity of antiserum produced against the DS-maltE fusion.

Before expression and purification of fusion proteins, the nucleotide sequences of the 5' and 3' vector-insert junction sites were determined by using a primer complementary to pMAL-c2 at about 40 bp upstream of the maltE–polylinker junction site, together with the reverse and universal M13 sequencing primers for pUC19 constructs. Dideoxy sequencing (Sanger et al., 1977) was performed using the Sequenase kit (US Biochemical).

**Purification of MBP fusion proteins and production of antisera.** Expression and purification of MBP fusion proteins were essentially as described in the manufacturer's manual. After column purification, proteins were concentrated using Ultrafree-MC microcentrifuge filters, resuspended in PBS, emulsified with an equal volume of Freund's complete adjuvant (Sigma) and subcutaneously injected into a rabbit. Injections were repeated after a week using incomplete adjuvant. A pre-immune bleed was made before the first injection and samples were taken 17 days after the last injection. Antisera were prepared using the method described by Harlow & Lane (1988). Antiserum to whole RTSV particles was kindly provided by H. Hibino (IRRI, Philippines).

**Direct double-antibody ELISA.** ELISA was performed as described by Tijssen (1985). For visualization of the immunological reaction, anti-rabbit IgG–horseradish peroxidase conjugate was used and the presence of the enzyme was revealed by the colour reaction with o-phenylenediamine (Sigma).

**SDS–PAGE and Western blots.** SDS–PAGE was as described in Sambrook et al. (1989) and by Schägger & von Jagow (1987). Gels were silver-stained as described by Blum et al. (1987).

In Western blots (Blake et al., 1984), the immunological reaction was visualized using anti-rabbit IgG–alkaline phosphatase conjugate and the presence of the enzyme was revealed by the colour reaction with Sigma FAST NBT/BCIP tablets.

**Immunogold electron microscopy.** A virus preparation (10 μl) was air-dried onto pyroxilin (4%)–carbon-coated gold grids previously treated with one drop of Butvar solution and incubated on 10 μl antisera diluted in blocking buffer (10 mM-Tris–HCl, pH 7.4, 0.9% NaCl, 0.05% PEG 2000, 3% BSA) for 1 h at room temperature. Anti-rabbit serum conjugated with 10 nm gold particles (Sigma) diluted in blocking buffer for 1 h at room temperature.
### Sequencing of CP3 of the Indian isolate

First-strand cDNA synthesis of the Indian isolate of RTSV was performed using random hexanucleotides (Sigma). For PCR of the region covering, and downstream of, CP3, a 5' primer CATGATGTGCAGGCTGGCTGGCGAAACGGTATCTGCGT (nt: 3659 - 3675) and a 3' primer CATACTTCCCTCGAAAACGTCTAGGC (nt: 4753 - 4728) were used. The 1.2 kbp PCR product was gel-purified, cloned in EcoRV-cut T-tailed pBluescript SK(+) (Stratagene) and sequenced by fluorescent dye terminator cycle sequencing, using the PRISM Ready Reaction kit on an ABI 373A automatic sequencer according to the manufacturer's instructions.

### Data analysis

Molecular mass estimation and quantitative analysis were performed using the IMAGE QUANT program (Molecular Dynamics). Sequences were analyzed using programs from the University of Wisconsin Genetic Computer Group (UWGCG; Devereux et al., 1984) and from the Staden package (Staden, 1982).

### Results and Discussion

**CP3 is a dominant antigenic determinant on the surface of RTSV**

First-strand cDNA synthesis was on RNA extracted from semi-purified RTSV (Philippine isolate) using primer 10 (Fig. 1). Double-stranded cDNAs to each CP were synthesized from this first-strand DNA using primer pairs 1 + 2 for CP1, 3 + 4 for CP2 and 5 + 6 for CP3 (Fig. 1), cloned into pMAL-c2 and expressed, and the fusion proteins were purified on affinity columns. MBP–CP fusion proteins themselves were used for immunization of rabbits, as attempts to use factor Xa to release the CP moieties resulted in degradation of the CPs. In control experiments, no signal was detected when Western blots of protein extracts from healthy and virus-infected plants were probed with antiserum to the MBP, indicating that there was no cross-reactivity of MBP antibodies and plant proteins.

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**Fig. 1.** Genome organization of RTSV (from Shen et al., 1993) and sequences of the primers. The boxes represent the polyprotein and the short ORFs (sorfs), with the line at each end showing the non-coding part of the viral RNA, the 5' end being labelled NTR (non-translated region). Various features are identified in the polyprotein: CP1–3, coat proteins 1–3; DS, the downstream region described in the text; NTP, the nucleotide triphosphate binding domain; PRO, the protease domain; POL, the RNA-dependent RNA polymerase domain; the putative genome-linked protein VPg and the polyA are indicated at the 5' and 3' ends of the RNA respectively; ?, function unknown. The first and second lines show nucleotide and amino acid positions which are mentioned in the text. The arrows are the sites of primers used for PCR, which are detailed below. The bold letters show restriction endonuclease cutting sites and the double underlines indicate the translational stop codons added in the primers. The nucleotide numbers of the 5' and 3' ends of the primers are based on the sequence of Shen et al. (1993). The actual sequences of the primers are those determined by Zhang et al. (1993) and differ slightly from the published sequence of Shen et al. (1993).
Fig. 2. SDS–PAGE and Western blot analysis of MBP–CP fusion proteins and CPs of the Philippine isolate. (a) Coomassie Brilliant Blue-stained gel after electrophoresis of 1 µg MBP-CP1 (track 1), MBP-CP2 (track 2) and MBP-CP3 (track 3). (b) Western blot of MBP-CP1 (track 1), MBP-CP2 (track 2) and MBP-CP3 (track 3) (each 100 ng protein) probed with antiserum to whole RTSV particles. (c) Silver-stained gel after electrophoresis of virus preparation (A₂₆₀ 1·0) made using SP249 cellulase. Tracks: 1, 10 µl virus; 2, 2 µl virus; 3, 0·4 µl virus. (d) Western blot of the same virus preparation (0·2 µl) probed with antiserum to RTSV particles. Molecular masses are shown.

The yield of all three fusion proteins purified from 100 ml of *E. coli* JM83 cells (OD₅₅₀ 1·0) was 2–3 mg.

After PAGE and Coomassie Blue staining of the gels, uncleaved fusion proteins showed as major bands of expected sizes with molecular masses of 66, 65 and 78 kDa for MBP–CP1, MBP–CP2 and MBP–CP3, respectively, together with smaller proteins (Fig. 2a). To obtain information about these smaller proteins, Western blots were probed with antiserum either against whole virus particles (Fig. 2b) or against MBP (data not shown). No specific signals were detected on analysing proteins from cells transformed with vectors other than pMAL-c2 or from non-transformed cells (data not shown). Thus, the extra bands of MBP–CP fusion products appear to arise from either premature transcription–translation termination or non-specific proteolytic cleavage.

The antiserum against the three CPs specifically recognized their respective CP (Figs 2b and 3), with no evidence of cross-reaction. Polyclonal antiserum against each CP was titrated in ELISA and Western blots using a highly purified RTSV preparation (A₂₆₀ 1-0). In an ELISA using 1·5 µl of a virus preparation, anti-CP1 and -CP3 antiserum had titres of 10⁻³ and anti-CP2 antiserum had a titre of 6·7 × 10⁻⁴. In Western blots, the CP band from 10 µl of the virus preparation was detected by antiserum to CP1 at a dilution of 5 × 10⁻⁴, that to CP3 at a dilution of 5 × 10⁻⁵, and that to CP2 at a dilution of 5 × 10⁻⁶. Thus, both tests showed that the antiserum to CP2 had the highest titre.

The antiserum to whole virus particles (Philippine isolate) gave a stronger signal in reaction with CP3 than with CP1 or CP2 (Fig. 2b), whereas Coomassie Blue staining (Fig. 2a) indicated that the loadings were similar. Thus, the stronger signal of the CP3 fusion (Fig. 2b, track 3) could be due to either CP3 being in excess or it being the major antigenic determinant on the virus particle. Scanning of a silver-stained PAGE gel of virus purified using SP348 cellulase (see below) (Fig. 2c) indicated that all three CPs were in equimolar amounts in the virus particle, assuming that they stain equivalently. This confirms the suggestion of Zhang et al. (1993a) of equimolarity of the CPs based on Coomassie Blue staining. The immunodominance of CP3 was supported by ELISA using 200 ng of recombinant fusion proteins in which the antiserum against whole virus had a titre of 10⁻³ against CP1, of 5 × 10⁻⁴ against CP2 and of 2 × 10⁻⁴ against CP3.

Further evidence on the immune presentation of the three CPs was obtained from immunogold electron microscopy. For this, two different highly purified RTSV preparations (concentrations of virus: A₂₆₀ 1·0 and A₂₆₀ 0·6) were analysed using a double-antibody sandwich assay, with the primary antibody being anti-CP1 (1:50), anti-CP2 (1:500), anti-CP3 (1:250) or pre-immune antiserum and the secondary antibody being gold-labelled anti-rabbit antiserum. In total, an area of 20 µm² (fields of 5 µm² from four different parts of the grid) was observed for each experiment (Table 1). Table 1 clearly shows that more gold bound to virus particles in the presence of antiserum to CP3 than in the presence of CP1 and CP2. This confirms the previous results, indicating that CP3 has a dominant role in the antigenicity of the virion.

Based upon genome position, RTSV CP3 can be considered to be the structural equivalent of virion protein 1 (VP1) of animal picornaviruses or the small CP of comoviruses (see Goldbach, 1987; Wimmer et al., 1993). The capsid protein folding pattern is widely conserved among spherical RNA viruses of eukaryotes (Rossmann & Johnson, 1989; Harrison, 1990). The basic structure of the capsid protein is an eight-stranded, anti-parallel β-barrel, the strands of which are connected by amino acid loops exposed on the outer surface of...
Table 1. Immunogold labelling of RTSV (n = 3)
The results show the mean number of gold particles per 20 μm². Figures in parentheses are gold particle no./virus particle no. expressed as a percentage.

<table>
<thead>
<tr>
<th>Virus preparation</th>
<th>$A_{260} \cdot 1.0$</th>
<th>$A_{260} \cdot 0.6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP1 antiserum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bound-to-virus gold particles</td>
<td>5.3 ± 1.1 (4.8%)</td>
<td>8.5 ± 1.0 (13.8%)</td>
</tr>
<tr>
<td>Number of virus particles</td>
<td>1100 ± 80</td>
<td>61.5 ± 8.2</td>
</tr>
<tr>
<td>CP2 antiserum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bound-to-virus gold particles</td>
<td>14.5 ± 2.0 (16.0%)</td>
<td>8.7 ± 1.3 (17.8%)</td>
</tr>
<tr>
<td>Number of virus particles</td>
<td>90.5 ± 11.5</td>
<td>49.0 ± 4.5</td>
</tr>
<tr>
<td>CP3 antiserum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bound-to-virus gold particles</td>
<td>127.8 ± 9.6 (120.5%)</td>
<td>65.0 ± 9.0 (114.5%)</td>
</tr>
<tr>
<td>Number of virus particles</td>
<td>1060 ± 80</td>
<td>56.7 ± 2.25</td>
</tr>
</tbody>
</table>

The virion. In picornaviruses, these loops often contain neutralizing antigenic sites (see Smith et al., 1995; Brown, 1995). One or more of the loops in VP1 of many picornaviruses is antigenic (see Chapman et al., 1990) and one in foot-and-mouth disease virus VP1 is the most immunodominant (Brown, 1995). Similarly, the C terminus of the small protein of bean pod mottle comovirus, which is analogous to VP1, plays a dominant role in the antigenicity of the virus (Joisson & van Regenmortel, 1991).

Modification of CP3

Blots of proteins from a highly purified RTSV preparation, made using driselase, and from sap from 2–3-week-old rice plants with or without tungro disease symptoms were probed with antiserum against each RTSV CP (Fig. 3). These antisera detected CP1, CP2 and CP3 with expected molecular masses (22.5, 22.0 and 33 kDa) in virus preparations (Fig. 3a, b, c, track 1), and also detected CP1 and CP2 in sap extracts (Fig. 3a, b, track 2). The extra band of about 45 kDa in CP2 of virus preparations (Fig. 3b, track 1) is considered to be a dimer. The CP3 antiserum also detected several other proteins (40–42 kDa) in purified preparations (Fig. 3c, track 1) and only these other proteins, and not the 33 kDa protein, in extracts from infected plants (Fig. 3c, track 2). Apparently the CP3 33 kDa protein is a product of virus purification.

The CP3-related 40–42 kDa proteins could be products of alternative cleavage of the RTSV polyprotein. Since the bands were not detected by the CP2 antiserum (Fig. 3b, tracks 1 and 2), they could not be due to faulty cleavage between CP2 and CP3. To investigate the possibility of the absence of cleavage at the expected site at the C terminus of CP3, a cDNA to a 13 kDa region of the RTSV polyprotein downstream of CP3 (from amino acids 1359 to 1483) (see Fig. 1) was synthesized by PCR using primers 7 + 9 and cloned into pMAL. This was expressed and an antiserum was produced against the fusion protein. The specificity of this antiserum was confirmed by it detecting the E. coli-expressed 22 kDa lacZ in-frame fusion with the DS region [cDNA synthesized using primers 8 + 9 (Fig. 1) and cloned into pUC18; data not shown]. Since the antiserum to MalE alone did not react with the DS-lacZ fusion, the serum produced to the DS fusion specifically detected the DS domain. The DS antiserum did not detect the 40–42 kDa proteins in blots of sap from infected plants (data not shown). Furthermore, the antiserum to virus prepared with driselase, and likely to contain the 40–42 kDa proteins, did not detect
the E. coli-expressed DS fusions (data not shown). Thus, the larger forms of CP3 are not the result of faulty cleavage and are likely to arise from post-translational modification.

The standard purification procedures (Cabauatan & Hibino, 1988; Jones et al., 1991) involve incubation of extracts from infected plants with a cellulase, driselase or Celluclast to release virus particles from vascular bundle cells. To assess whether the 33 kDa CP3 resulted from this digestion, Western blots were made of viral proteins from preparations made with and without using cellulolytic enzymes (Fig. 4a, tracks 2 and 3). It is clear from this that incubation with Celluclast led to the change from 40–42 kDa to 33 kDa CP3; this change was complete with an experimental cellulase (SP348) (Fig. 4a, track 1) and thus this enzyme was used to investigate the stoichiometry of the three CPs (Fig. 2c). To investigate further the change from the 40–42 kDa to the 33 kDa form, the effects of various treatments on the ratio of the two forms were examined (Fig. 4b; see also Fig. 6). Onozuka RS cellulase is also very efficient at converting the larger to the smaller form (Fig. 4b, track 5) but reduces the amount of protein, probably by proteolysis. The suppression of the activity of Onozuka RS cellulase by heating (Fig. 4b, track 4) indicated that it was
enzyme activity that caused the reduction in size and amount of CP3. Activity causing this effect was also present in plant sap (Fig. 4b, cf. tracks 1 and 2 with track 6), but it resulted in several bands in the 33 kDa region. Onozuka RS cellulase had less effect on crude extracts than on purified virus (Fig. 4b, cf. tracks 3 and 5).

At this stage, the nature of CP3 modification is not understood. There are various ways in which virus proteins are post-translationally modified. Electron microscopy observation of virus preparations with different CP3 forms did not reveal any obvious differences. The CPs of several plant viruses form an association with ubiquitin (Hazelwood & Zaitlin, 1990). Western blots of the 40-42 kDa form of CP3 did not reveal anything when probed with anti-bovine ubiquitin antibody (Sigma), which recognizes yeast ubiquitin (Sigma) (data not shown).

The modification seems not to be due to protein–protein interactions as it gives a heterogeneous population of molecules and it modifies CP3 in such a way that it does not stain with silver nitrate (Fig. 5b, track 1) or Coomassie Blue yet is detected by CP3 antiserum (Fig. 5c, track 1). In tests for glycosylation, gels were stained with Schiff's reagent or viral proteins were labelled with hydrazide conjugate (Wilchek & Bayer, 1987), or were deglycosylated by β-elimination (Downs et al., 1973), acid hydrolysis (Ausubel et al., 1987) or with trifluoromethanesulphonic acid (Hakimumuddin et al., 1987). None of these treatments gave any evidence of glycosylation. Similarly, there was no change in the mobility of the 40–42 kDa bands after treatment with ribonuclease or with ethanol–ether or chloroform–methanol, which would remove lipids (Mills et al., 1989) (data not shown).

The electrophoretic mobilities of CP1 and CP2 were similar for all isolates tested. CP3-related 40–42 kDa proteins of the Indian isolate obviously differ from the others in that they migrate more slowly, giving an apparent molecular mass about 2 kDa greater than that of the Philippine, Thai and Malaysian isolates (Fig. 5a).

The difference between the Indian and south-east Asian isolates is more obvious if virus particles purified using the standard procedure with Celluclast are analysed (Fig. 5b, c); the 33 kDa CP3 is absent from the Indian isolate.

Incubation of virus preparations of both isolates with several cellulytic enzyme mixtures (Fig. 6) resulted in different patterns of 33 kDa/40–42 kDa CP3-related proteins: e.g. treatment with SP249 resulted in total conversion to the 33 kDa form in the Indian isolate but only partial conversion in the Philippine isolate (Fig. 6, tracks 1 and 2), in contrast with Celluclast having more effect on the Philippine isolate than the Indian isolate (Fig. 6, tracks 5 and 6). This suggests that Philippine and Indian isolates might have a different kind of modification.

The difference between the Philippine and Indian isolates in the size of the 40–42 kDa CP3s is reflected in the 33 kDa protein, which in the Indian isolate migrates as a 35 kDa protein (Fig. 5d). As the difference in size is not affected by removal of the modification of the CP3, it must be due to either differences in cleavage sites, an insertion in the Indian isolate sequence or differences in amino acid sequence. The CP3 region of the Indian isolate was cloned and sequenced and compared with that of the Philippine isolate. There are 13 amino acid substitutions, six being conservative (S → N at position 1087, S → C at 1181, E → D at 1296, D → E at 1297, T → S at 1301 and I → V at 1360) and seven non-conservative (F → L at 1218, I → T at 1292, N → D at 1294, T → A at 1328, T → E at 1341, Q → H at 1346 and N → D at 1372); there were no deletions or additions. Thus, the CP3 of the Indian isolate contains substitutions which result in two more acidic residues (aspartic acid) and one more basic residue (histidine) than in the Philippine isolate; these give one more net negative charge, which would be expected to increase the relative mobility. The actual C terminus of CP3 has not yet been determined. By analogy with picornaviruses, it could be processed by either a 2A- or 3C-type protease (Wimmer et al.,

Analysis of RTSV isolates from different geographical regions

To see if there was any serological variation between the individual CPs of isolates of RTSV from the Philippines, Malaysia, Thailand and India, Western blots were probed with a mixture of antisera to all three CPs (Fig. 5a). In all, 67 plants were analysed (Fig. 5a shows some of these) but no differences were observed in the reactivity to the different CP antisera, suggesting no major serological differences between them.


Shen, P., Kaniewska, M., Smith, C. & Beachy, R. N. (1993). Thus, it is not possible to say if the difference in size between the Indian and Philippine CP3s is due to differences in the C-terminal processing site.

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References


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