Purification and Partial Characterization of Rice Transitory Yellowing Virus

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ABSTRACT

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Rice transitory yellowing virus (RTYV) was purified by procedures that included steps of density gradient centrifugation in 30% Percoll and subsequent fractionation on Sepharose CL-4B column for removal of Percoll particles. Electrophoresis of sodium dodecyl sulfate-disrupted virus in polyacrylamide gels yielded three major proteins of 90K, 63K, and 32K daltons and two minor proteins of 200K and 43K daltons. These represent the virion proteins L, G, N, NS, and M, respectively, in decreasing order of their molecular weights. Antisera prepared to RTYV and potato yellow dwarf virus were tested by enzyme-linked immunosorbent assay and by immunoblotting with homologous as well as heterologous antigens. There was no serological relationship between the two plant rhabdoviruses. When examined in the electron microscope by negative staining, unfixed particles of purified RTYV were bullet shaped, measuring 124 × 93 nm. These estimates are consistent with previous size measurements for particles in dip preparations.

Additional keywords: electron microscopy, potato yellow dwarf virus, rhabdovirus, serological relationship, virion proteins.

Rice transitory yellowing was first found in Taiwan in early 1960s and shown to be transmitted by the rice green leafhopper *Nephotettix nigropictus* (Stål) and two related species (9,10,25). Multiplication of rice transitory yellowing virus (RTYV) in vector insects was demonstrated by serial injections of leafhoppers with extracts prepared from viruliferous insects (24). In leaf dips and thin sections of infected rice plants, the virus had a bullet-shaped particle morphology, a basis for its grouping with other rhabdoviruses infecting plants (19–21,31).

Until 1977, RTYV had not been described outside Taiwan. Recently, it has been reported to occur in the Ishigaki and Okinawa islands of Japan (27,42), on wet-season rice in northern Thailand (22) and, under the name of yellow stunt, in some rice-growing areas in the central and southern parts of China (8,16).

The cellular and intracellular distributions of RTYV have been studied extensively by electron microscopy of sectioned rice leaf tissues. Particles of RTYV were largely confined to perinuclear spaces in phloem cells (7). To a lesser extent, virus particles could

also be found in other cell types and in the cytoplasm (33). Usually, cells of stem and root contained fewer particles than leaf cells (33).

In this paper, we report the purification of RTYV by a procedure involving the use of Percoll density gradient, the protein composition of the virus and present evidence of a lack of serological relationship between RTYV and the *sanguinolenta* strain of potato yellow dwarf virus (PYDV) (4). Preliminary reports have appeared elsewhere (12,13).

MATERIALS AND METHODS

Viruses and plants. The source of RTYV was described previously (6). The *sanguinolenta* isolate of PYDV (4) was obtained from Dr. H. T. Hsu (USDA Agricultural Research Service, Beltsville, MD). RTYV was propagated and maintained in rice cultivar Tainan 5 plants and PYDV in *Nicotiana rustica* L. For maintenance of RTYV, rice plants were inoculated at the three- to four-leaf stage with a leafhopper (*Nephotettix cincticeps*) vector. *N. rustica* plants were inoculated mechanically with PYDV when the plants had three to five fully expanded leaves. Before inoculation the plants were kept in the dark for 18–24 hr. All test plants were grown in the greenhouse where temperatures fluctuated between 25 and 30 C in the spring and fall when most experiments were conducted.

Assays of virus infectivity. RTYV was assayed for infectivity by either of the following ways: 1) Virus samples were injected into fourth or fifth instars of *N. cincticeps* and the insects kept in a group for 10–14 days on healthy rice plants in a 28 C growth chamber. Afterward, the insects were transferred individually to a series of rice seedlings grown to the two- to three-leaf stage in test tubes for inoculation. The inoculated plants were then transplanted to pots and placed in the greenhouse for symptom development. 2) Virus samples were rubbed onto leaves of *N. rustica* to produce chlorotic local lesions, which developed 38–45 days after inoculation (11).

Purification of viruses. RTYV was purified from symptomatic leaves harvested from greenhouse-grown rice plants 3–6 wk after inoculation. The leaves were finely cut in the cold and wet with 3 volumes (w/v, 1 g:3 ml) of extraction buffer, which consisted of 0.1 M phosphate, pH 7.4, 0.5% 2-mercaptoethanol, and 10 mM EDTA. Usually, about 100 g of diseased leaves were used as starting material and homogenization was done with a Polytron (Kinematica, Switzerland). The homogenate was strained through cheesecloth and centrifuged twice at 5,000 *g* for 5 min each. The second supernatant which appeared light green was vacuum-filtered through a 0.5- to 0.7-cm Celite cake in a 12.5 cm Büchner funnel. Percoll (Pharmacia, Uppsala, Sweden) was added to the filtrate at a ratio of 3 ml of Percoll/7 ml of filtrate. After brief stirring, the virus sample was centrifuged at 22,500 rpm for 30 min in a Beckman SW 28 rotor. The virus zone was withdrawn, diluted with a buffer consisting of 10 mM phosphate, pH 7.4, 0.05% 2-mercaptoethanol and 1 mM EDTA, and centrifuged at 25,000 rpm for 30 min in an SW 28 rotor. This step yielded a gelatinlike clear pellet of Percoll particles overlaid with virus. The latter was removed from the tubes with a syringe and suspended in 0.01 M phosphate buffer as above. This was followed by fractionation on a Sepharose CL-4B (Pharmacia) column, 54 × 1.2 cm, previously equilibrated with same buffer and regulated at an elution rate of 20 ml/hr to remove Percoll. The fractions were scanned with an LKB Uvicord I absorption meter for light absorbance at 254 nm. Early fractions following the void volume that absorbed light strongly were collected. They were either examined in an electron microscope or further subjected to centrifugation at 22,000 rpm for 60 min in 10–40% sucrose density gradients formed in suspending buffer in an SW 41 rotor. The virus band was drawn, diluted, and centrifuged at 27,000 rpm for 30 min in a Beckman type 30 roter.

An alternative purification procedure for RTYV that gave equally satisfactory results involved substitution of Tris-HCl for phosphate buffer at the same pH and molarities for extracting and suspending the virus. One repetition of Percoll density gradient centrifugation was necessary when the starting material was from aged plants and the virus samples from the first Percoll density gradients contained greenish contaminants.

PYDV was purified by a procedure that we have previously used (36). Leaves harvested from infected *N. rustica* plants 14–20 days after inoculation were homogenized in an extraction buffer composed of 0.1 M Tris-HCl, pH 8.4, 0.01 M magnesium acetate, 0.04 M Na_2SO_3, and 1 mM $MnCl_2$ (30). The homogenate was strained through cheesecloth and centrifuged at 3,000 *g* for 10 min. The supernatant was clarified by adding K_2HPO_4 and $CaCl_2$ each to a final concentration of 50 mM with stirring and was centrifuged again at low speed. The supernatant was then centrifuged through a 20% sucrose pad at 27,000 rpm for 45 min in a Beckman type 30 roter. The virus pellet was suspended in the same buffer as above and the suspension subjected to rate zonal centrifugation at 25,000 rpm for 20 min in 5–30% sucrose gradients. The virus band was removed and further subjected to quasi-equilibrium centrifugation in 30–60% sucrose gradients for 90 min at 25,000 rpm. Both gradients were prepared in Beckman SW 28 tubes in a buffer that was a 10-fold dilution of the extraction buffer, adjusted to pH 7.0.

Isolation of PYDV and RTYV nucleocapsids. The procedure used to isolate nucleocapsids of PYDV and RTYV was essentially same as that for virus purification, except that Triton X-100 was included in the final step to remove the viral envelope. The detergent was added to the suspension of purified virus at a final concentration of 2% and the mixture stirred for 10 min at 4 C. The disrupted virus preparation was fractionated on a 10–40% sucrose density gradient in suspending buffer by centrifugation for 90 min at 36,000 rpm in a Beckman SW 41 rotor. The light scattering band was collected, diluted with buffer, and pelleted at 32,000 rpm for 2 hr in a Beckman type 50 rotor.

Polyacrylamide gel electrophoresis. Purified RTYV and PYDV samples were prepared for polyacrylamide gel electrophoresis (PAGE) by solubilization in a buffer consisting of 0.06 M Tris-HCl, pH 8.8, 2% SDS, 2 mM EDTA, 10% Ficoll, 5 mM dithioerythritol, and 0.001% bromophenol blue (35). The mixture was boiled for 3 min. Electrophoresis was performed in a 7.5–15% linear polyacrylamide gradient gel with Laemmli's discontinous buffer system (34). The gels were stained with silver nitrate (39). Glycoproteins were detected by the periodic acid-Schiff staining procedure (17). As molecular weight markers, myosin (M_r 200,000), β-galactosidase (116,000), phosphorylase b (92,500), bovine serum albumin (66,000), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400) were used.

Serology. Antiserum to RTYV was produced in rabbits by four intramuscular injections made at weekly intervals. For each injection about 0.5 ml of virus sample, representing the total harvest of a purification run from a starting material of 60–100 g, was given. Before injection, the virus sample was emulsified with an equal volume of Freund's incomplete adjuvant. The antiserum obtained from blood collected 10 days after the last injection had a titer of 1:16 in double diffusion tests against crude sap of infected rice leaves (R. J. Chiu, *unpublished*). Antiserum to PYDV was also produced in a rabbit by a similar procedure, but Triton X-100 disrupted virus was used as immunogen. This antiserum had a titer of 1:32 in double diffusion tests against crude sap of infected leaves of *N. rustica*.

ELISA tests were performed for purified virus preparations as well as for viruses in crude extracts prepared from their specific host plants. Infected leaves were homogenized in distilled water and diluted in a coating buffer (0.05 M sodium carbonate, containing 0.02% NaN_3, pH 9.6) and the extracts centrifuged at low speed. The polystyrene microtiter plates (Dynatech Labs., Alexandria, VA) were coated with dilutions of antigens, treated with primary antiserum, reacted with an alkaline phosphatase conjugate of goat anti-rabbit IgG (Sigma). After washing, ρ-nitrophenyl phosphate was added for color development according to the standard procedure (37). Readings were made with a Bio-Tek Microplate reader (Bio-Tek Instruments, Burlington, VT).

For Western blotting, viral proteins obtained after electrophoresis were transferred from polyacrylamide gels to nitrocellu-

lose paper with a Bio-Rad Electroblotter providing a current of 200 mA for 6 hr (45). The buffer was the same as that used for PAGE, except that it contained 20% (v/v) methanol. The nitrocellulose paper containing transferred proteins was cut into vertical strips, each with paired samples of RTYV and PYDV. Protein bands were detected with colloidal gold by a procedure described previously (36). For immunolabeling, the remaining strips that also contained paired samples of virus proteins were first blocked with 3% bovine serum albumin (BSA) in 0.1 M phosphate-buffered saline (PBS), pH 7.2, for 1 hr at 37 C. Either RTYV or PYDV antiserum was then added to the blocking solution to a final dilution of 1:500, and the cellulose strips were incubated for 2 hr at room temperature. After washing thoroughly in PBS containing 0.3% (w/v) Tween 20, blots were again blocked with PBS containing 3% BSA, and subsequently incubated for 2 hr with a horseradish peroxidase conjugate of goat anti-rabbit IgG (Sigma). The immunoblots were next washed in PBS-Tween, rinsed in distilled water and treated with a peroxidase substrate (60 mg of 4-chloro-1-naphthol dissolved in 20 ml of methanol, added to 100 ml of PBS, containing 40 μl of 30% H_2O_2).

Electron microscopy. RTYV sampled at various purification steps was mixed with an equal volume of 2.5% potassium phosphotungstate (PTA), pH 7.0, containing 0.05% BSA and the mixture transferred to carbon-coated Formvar membrane on grids. Rarely, uranyl acetate, pH 4.3, was used at 2% to stain virus and the degradation forms. The stained specimens were examined in a JEOL 100B electron microscope at 80 kV.

RESULTS

Purification of RTYV. Early attempts to purify RTYV by the procedures developed for PYDV (4,26), Sonchus yellow net virus (30), and other plant rhabdoviruses (2,31,41) failed. These procedures employed an extraction medium that contained one or more kinds of divalent cations, most notably Mg^{2+}, to effect virus stabilization (5). The presence of Mg^{2+} in extraction medium for RTYV, however, led to serious loss of virus during subsequent low-speed centrifugation of the leaf extracts. In one of our experiments, virus was extracted from diseased rice leaves with a buffer system composed of 0.1 M phosphate, pH 7.4, and 0.5% 2-mercaptoethanol, which was amended with 10 mM EDTA, 10 mM DIECA, 10 mM $MgCl_2$, or no additive. The leaf extracts, clarified at 3,000 *g* for 5 min, gave a total of 329, 525, 10, and 74 lesions, respectively, on 10 inoculated leaves of *N. rustica*. Loss of RTYV in the last two treatments probably occurred during low-speed centrifugation as a result of aggregated particles. Such losses could be prevented to a large extent by incorporating suitable chelating compounds into the extraction medium. We routinely used EDTA for this purpose.

RTYV was purified successfully from infected rice leaves by a procedure that included the steps of density gradient centrifugation in Percoll and chromatographic separation on a Sepharose column as described in the Materials and Methods section. In Percoll gradients, virus particles sedimented to form an opaque zone near the bottom of the centrifuge tubes, while the host-derived green particulates were largely excluded from the gradient. Less effective exclusion was obtained when lower ratios of Percoll/buffer were used. The contaminating Percoll particles were then separated from virus particles by a passage through Sepharose CL-4B column. Characteristic bullet-shaped particles of RTYV were detected in fractions immediately following the void volume. Normally, the virus particles suffered little or no apparent damage at this stage. Damage often occurred when the virus was subjected to sucrose density gradient centrifugation. Complete removal of Percoll particles was not achieved even by carrying the purification procedure to completion. Yields of purified virus were generally

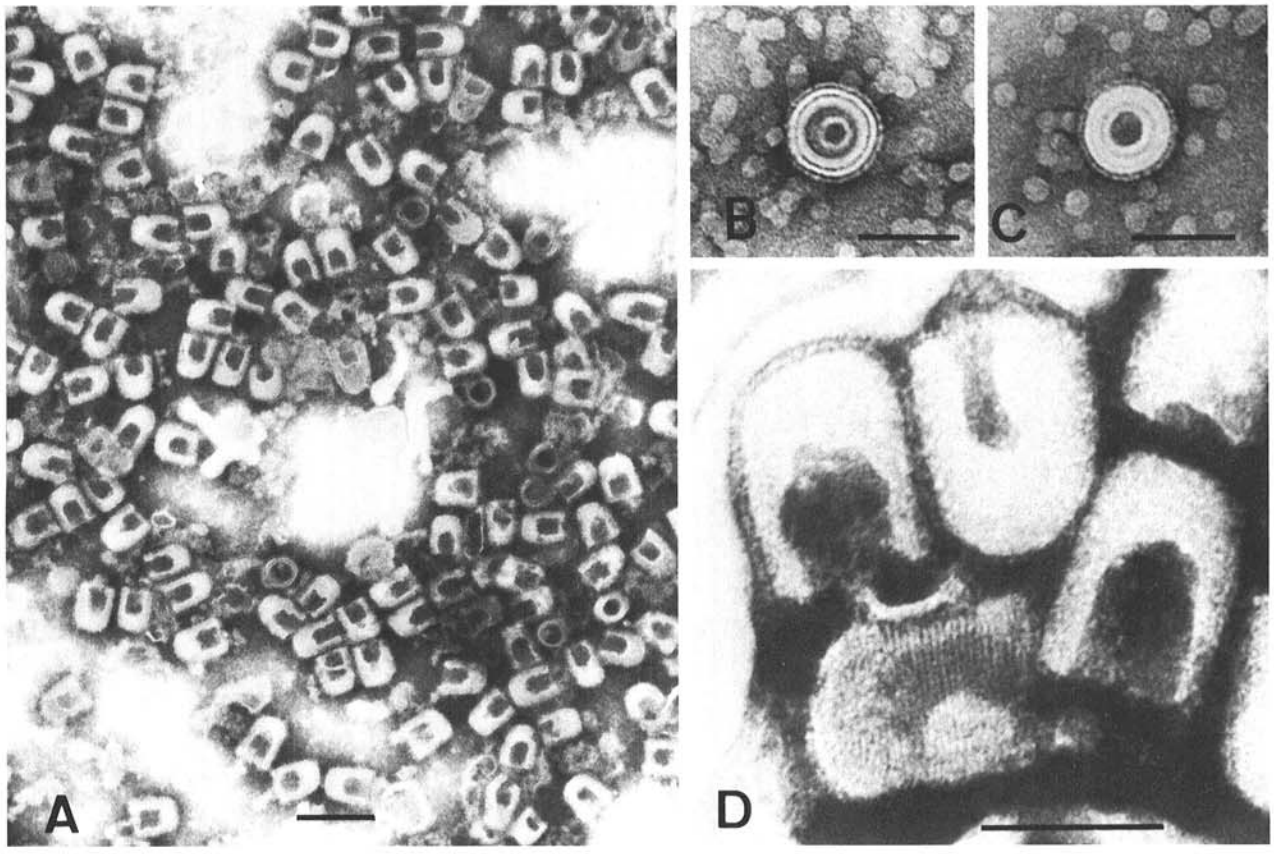

Fig. 1. Electron micrographs of purified rice transitory yellowing virus (RTYV) negatively stained with PTA. **A,** RTYV particles recovered from 10 to 40% sucrose density gradients after centrifugation at 60,000 *g* for 60 min. The virus samples had previously been subjected to density gradient centrifugation in 30% Percoll and fractionation on Sepharose CL-4B column. Contaminating Percoll particles are still evident in the micrographs. **B and C,** End-on view of two fragmented RTYV particles. **D,** RTYV particles sampled from the eluate of a Sepharose CL-4B column, showing surface structure and striations of the nucleocapsid core as revealed by PTA infiltration. Bar = 200 nm for A; 100 nm for B–D.

low and varied with the starting material. Typical purification runs gave about 140–850 μg of purified virus from 100 g of infected plant tissue, as estimated from the total protein content assayed according to Lowry et al (38), assuming that virion proteins of plant rhabdoviruses account for 70% of the particle weight (19,31). The yield of PYDV by our purification procedure ranged from 1 to 1.5 mg using the same amount of starting material.

In some purification trials, infectivity of virus samples taken at different stages was assayed either by injection of the vector leafhopper *N. cincticeps* or by mechanical inoculation of leaves of *N. rustica*. Infectivity was detectable in samples that had been fractionated through Sepharose column and in samples taken before Sepharose column fractionation (data not shown) but was not detectable in samples recovered from sucrose gradients. No attempt was made to compare quantitatively the infectivity recovery from different purification stages, because of the poor sensitivity of the assay methods.

Electron microscopy. Purified RTYV particles, negatively stained with PTA, were bullet shaped, with stain penetrating into the central hollow at the flat end (Fig. 1A and D). Rarely, the stain penetrated the envelope to reveal cross striations of the nucleocapsid (Fig. 1D). The majority (85%) of the enveloped particles measured 124 nm (range 67–130 nm) in length and 93 nm in width, not including the surface projections, which averaged 5.2 nm in height and 5 nm in width. The diameter of stained nucleocapsid core averaged 76 nm, with a center to center distance of 4.2 nm between two adjacent striations of 1.7 nm in width (Fig. 1D). The values for width of virus particles and nucleoprotein core are in fairly good agreement with those made previously on particles in dip preparations or in thin sections of infected rice leaves (7,43). The measured length of purified virus was consistent with that of virus particles in dip preparations, but was considerably shorter than that observed for particles in cells of infected host plants (7). This suggests that breakage of virions had occurred during virus extraction or in later steps of virus purification. End-on views of fragmented segments showed concentric ringlike structures, with the dimensions and inner details depending on where along the particle length the fragmented segments were derived (Fig. 1B and C). Properly oriented segments from the cylindrical part of the virus particles had a diameter of 90 nm, excluding the projections, and had four layers of electron-lucent ring. These layers, counting from the outer most, probably correspond to the projections, the envelope (a lipid bilayer membrane) seen as two layers (40), and the nucleocapsid of the virus, respectively. The number of projections that encircled the segments in end-on views was 28.

Purified particles of RTYV disintegrated readily when stored in suspending buffer at 4 C. Overnight samples stained with 2% uranyl acetate, pH 4.3, had ribbonlike structures protruding out from damaged particles (Fig. 2). By analogy with what has been observed in other rhabdoviruses (29,41,48), the extended ribbons represent unwound nucleocapsid. They had a width of 15 nm and cross striations repeated every 5 nm. No similar structure could be seen in samples stained with PTA.

Virion proteins of RTYV and PYDV. On SDS-PAGE, the protein profiles of the two viruses differed considerably (Fig. 3). Three major proteins with molecular weights estimated at 80K, 63K, and 32K Da, and two minor proteins of 200K and 43K Da, respectively, were resolved in the RTYV samples (Fig. 3, lane D). Infrequently, another minor protein of 52K was observed. This protein is probably derived from the host plant as it also appeared in electrophoresed material from healthy rice plants.

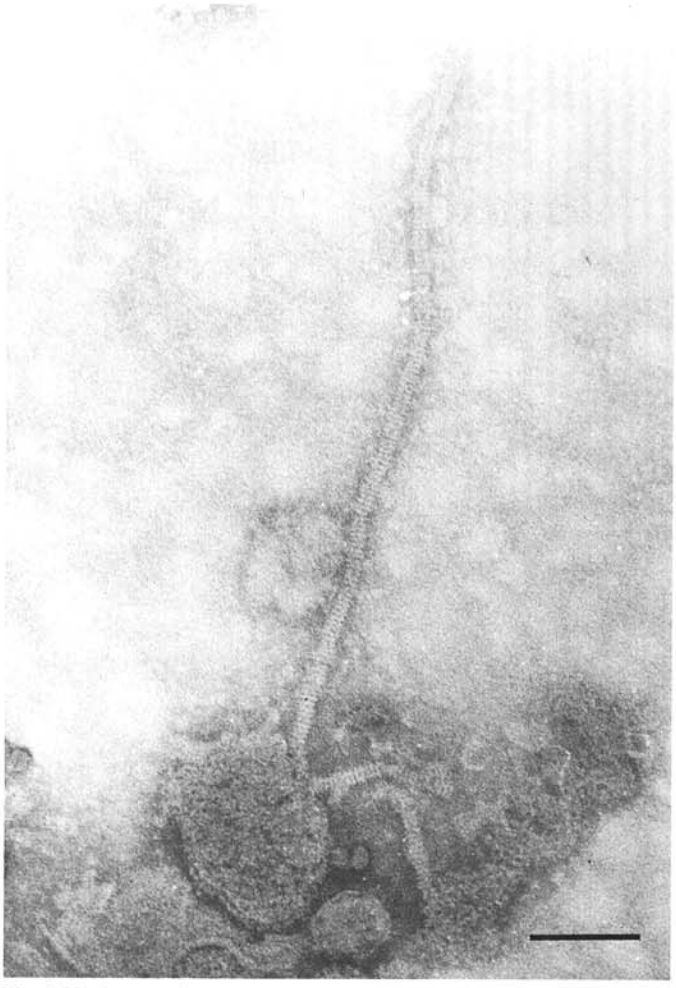

Fig. 2. Nucleoprotein strands released from rice transitory yellowing virus (RTYV) particles that had been stored overnight at 4 C. Uranyl acetate was used as a negative stain.

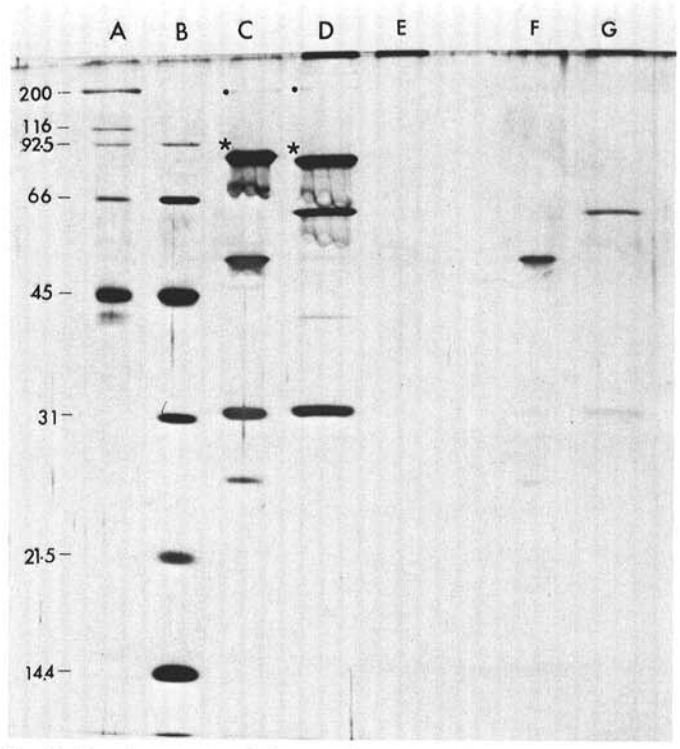

Fig. 3. Protein patterns of rice transitory yellowing virus (RTYV) and potato yellow dwarf virus (PYDV) and their nucleoprotein cores on SDS-7.5–15% polyacrylamide gel stained with silver nitrate. Conditions for electrophoresis are given in Materials and Methods section. Lanes A and B, molecular weight markers with M_r values (×1,000) indicated on the left side. Lane C, PYDV (In this lane, a bandlike colored area was an artifact.); Lane D, RTYV; Lane E, healthy rice plant material carried through the same purification procedure as for RTYV; Lane F, nucleoprotein core of PYDV; and Lane G, nucleoprotein core of RTYV. The L proteins of PYDV and RTYV are marked with a (.) and the G proteins with an (*).

The SDS-disrupted PYDV contained four major proteins of 84K, 52K, 31K, and 26K Da and two minor proteins of 190K and 48K, respectively (Fig. 3 lane C. In this lane, a bandlike colored area at 70K was an artifact possibly produced during electrophoresis). The 48K protein appears to be a degradation product that was not present in fresh virus preparations.

The 80K protein of RTYV and the 84K protein of PYDV gave a positive reaction after periodic acid-Schiff's staining and were completely removed from the virions after treating with Triton X-100. Thus, these proteins are designated G proteins since they correspond to the widely accepted criteria for rhabdovirus glycoproteins (46,47). The other three major proteins of PYDV (52K, 31K, and 26K) were detectable in the nucleocapsid core and are also believed to be viral components. These proteins appear to correspond to the N, M_1, and M_2 proteins (18,21,29,31). Two proteins, i.e., 63K and 32K, thought to represent the N and M proteins, respectively, were present in the nucleoprotein core of RTYV after Triton X-100 treatment. The 43K protein, which is probably the NS protein, was not detectable in the core prepared under our conditions. The 190K protein in PYDV and 200K protein in RTYV are believed to be the L protein of these rhabdoviruses (46,47).

Virion proteins of PYDV have been studied extensively (1,18,29,32). The only previous electrophoretic analysis of RTYV proteins has been conducted by Hayashi and Minobe (22) who described four major virion proteins with molecular weights estimated to be 92K (G), 72K (N), 43K (NS), and 28K (M), respectively. Among these, the 43K protein, though described as major, occurred in a considerably lower amount than the remaining three virion proteins as judged from the printed electrophoretic profile. The molecular weight values obtained by these authors differ somewhat from our determinations for the G, N, and M proteins and are possibly due to variations in electrophoretic conditions.

Serological tests. Antisera raised against RTYV and PYDV were tested against homologous as well as heterologous antigens by indirect ELISA. Using disrupted purified virus as a coating antigen, a reaction curve was obtained that related absorbance values (measured at 405 nm) to homologous antigen at a concentration range of 32–500 ng/100 μl for RTYV (Fig. 4B) and 4–500 ng/100 μl for PYDV (Fig. 4A). All dilutions of a purified PYDV sample failed to react with the RTYV antiserum, whereas the RTYV samples at relatively high concentrations were weakly reactive with PYDV antiserum (Fig. 4A and B).

Specific ELISA reactions were observed for RTYV in crude leaf extracts at dilutions up to 1:2,560 (w/v) with the homologous antibody (Fig. 4D) and for PYDV up to 1:5,120 (w/v) (Fig. 4C). Similar tests with leaf extracts from healthy host plants gave negative results for all dilutions of the two antisera (Fig. 4C and D). Here again, antiserum to PYDV showed a low level of reactivity with the heterologous antigens at low dilutions.

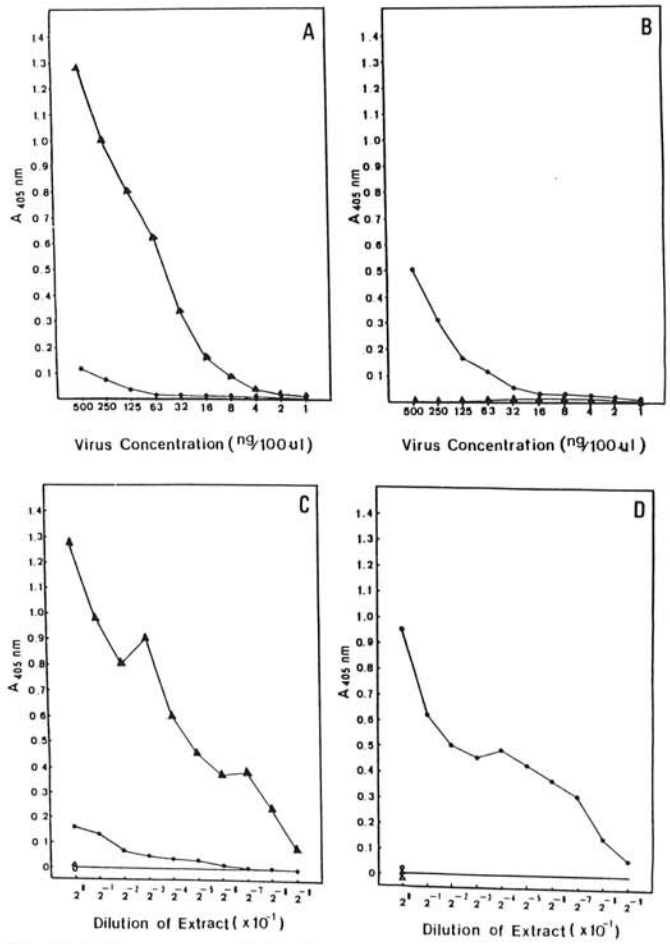

Fig. 4. Indirect enzyme-linked immunosorbent assay (ELISA) values measured at 405 nm for virus samples reacting with γ-globulin to, **A and C,** PYDV and, **B and D,** RTYV. **A and B,** Purified virus as coating antigen. ▲–▲, PYDV; ●–●, rice transitory yellowing virus (RTYV); **C and D,** leaf extract as coating antigen. ▲–▲, From PYDV-infected *Nicotiana rustica*; and △–△, from healthy *N. rustica*. ●–●, From RTYV infected-rice plants; ○–○, from healthy rice plants. One gram of leaf tissue was ground with 1 ml of distilled water to prepare each leaf extract. Nine milliliters of coating buffer was added to the homogenate to give a final dilution of 10^{-1}, from which a series of twofold dilutions was prepared as indicated. For ELISA tests on both purified viruses and leaf extracts, the γ-globulin was used at 1 μg/ml and the alkaline phosphatase conjugate of goat anti-rabbit IgG at 1:1,000 dilution. Readings were made 20 min after adding the enzyme substrate disodium p-nitrophenyl phosphate at 1 mg/ml.

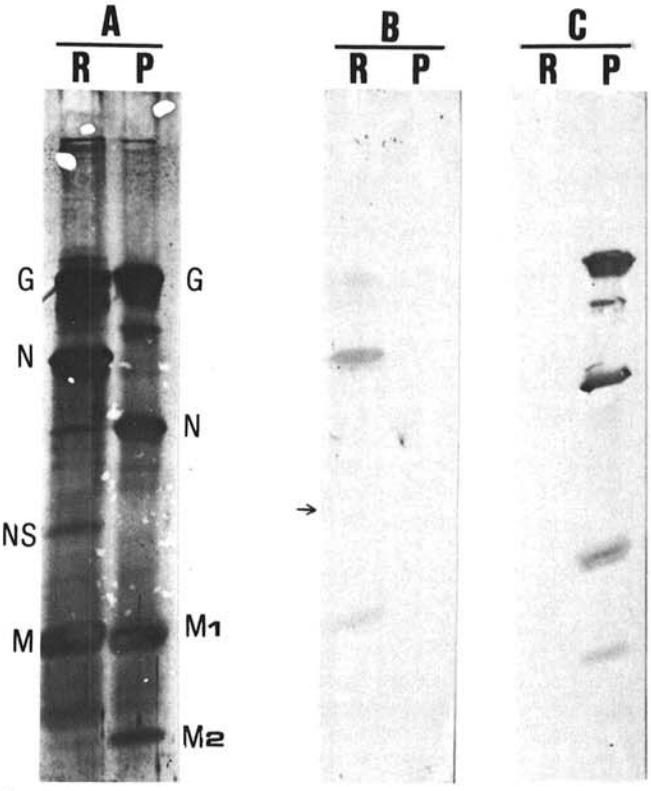

Fig. 5. Immuno-blotting tests of rice transitory yellowing virus (RTYV) and potato yellow dwarf virus (PYDV). **A,** Western blots of RTYV (lane R) and PYDV (lane P) proteins treated with colloidal gold to serve as reference. **B,** Western blots as in A but allowed to react with rabbit anti-RTYV serum, followed by treatment with horseradish peroxidase conjugate of goat anti-rabbit IgG. **C,** Western blots as in A treated with rabbit anti-PYDV serum. Subsequent treatments as in B. For both B and C, color was developed by adding 4-chloro-1-naphthol as substrate of the peroxidase. Arrow indicates the position of NS protein which is barely visible in the blots and did not appear when printed.

A lack of serological relationship between RTYV and PYDV was also evident from immuno-blotting tests. Successful transfer of the polyacrylamide gel-separated virion proteins was obtained for the G, N, NS, and M proteins of RTYV (NS was only weakly visible after treatment with homologous antiserum and may not appear in print) and for the G, N, M_1, and M_2 proteins of PYDV (Fig. 5). Protein L of the two viruses was not transferred or only inefficiently transferred, because of its large size and small quantity. The transferred virion proteins of either virus specifically reacted with the homologous antiserum only. No cross reactions were observed in the heterologous antigen-antibody combinations (Fig. 5).

DISCUSSION

Among members of the plant rhabdovirus group, RTYV is unique in that it infects both monocotyledonous and dicotyledonous plants. Infection of the rice plant, a natural host, requires *Nephotettix* leafhoppers as a vector (10), whereas infection of *N. rustica*, an experimental host, can be affected by rubbing the virus over leaf surface manually (11). Minute chlorotic lesions develop on the inoculated leaves but the virus fails to spread systemically. The reaction of *N. rustica* plants to RTYV inoculation appears to be erratic, making the new host unsuitable as a source for materials needed in virus purification. We, therefore, used infected rice plants for the purpose.

Purification of RTYV was accomplished by a procedure involving centrifugation in Percoll gradients in which virus particles were effectively concentrated in a visible opaque zone well separated from the majority of the host materials. Formation of virus aggregates was largely prevented by incorporation of EDTA into buffers for virus extraction. In the absence of this chelating agent in the extraction medium, most virus particles were lost after low-speed centrifugation. Aggregates developed that sedimented readily and failed to resuspend. In this respect, RTYV was similar to rabies virus (44) and sonchus virus (15), but behaved differently from PYDV (5), lettuce necrotic yellows virus (3,14), and several other plant rhabdoviruses (19,31) that require the presence of divalent cations in suspending medium for their stabilization.

Being an enveloped virus with a complex morphology, RTYV is rather stable in crude plant sap. Infectivity of the virus in extracts prepared from rice plants could be retained for 11 days at 2 C (23). There was little loss of infectivity after 24 hr at this temperature, when the virus was extracted and kept in a buffer that contained EDTA or DIECA and a reducing agent such as 2-mercaptoethanol. Therefore, difficulties in purifying RTYV as experienced in the early part of the present study stemmed from factors other than the instability of virus. These may include low virus content in host plants, poor virus release from plant tissues on extraction, and a ready tendency of virus particles to aggregate in vitro. The first two situations might be expected because the virus particles are mostly produced and distributed in the phloem cells, surrounded with a common membrane of host origin (7). We also experienced frequent failures in our attempts to purify RTYV in the winter months when the concentration of the virus seemed to be particularly low in the host plants.

Serological and protein composition comparisons were made between RTYV and PYDV because the two viruses share a number of common biological properties. Both viruses are sap transmissible to *N. rustica* where they produce similar chlorotic local lesions (4,11). Although rice is not a natural host of PYDV, injection of the virus into *N. cincticeps*, the vector of RTYV, and subsequent feeding on rice plants resulted in symptoms similar to those of RTYV (C. C. Chen and R. J. Chiu, *unpublished*). In addition, both viruses have been shown to assemble and accumulate in the perinuclear space (4,7,33). In spite of these similarities, the viruses were serologically unrelated and had different electrophoretic patterns for their proteins. The distant reaction of PYDV antiserum with RTYV antigen, but not of RTYV antiserum with PYDV antigen, at low dilutions by ELISA tests may need reexamination before it could be explained satisfactorily. One possible explanation at this moment would be that the PYDV antiserum may contain antibodies specific for certain epitopes possessed by both PYDV and RTYV. These epitopes, however, are not exposed if virus particles remain undisrupted, but are exposed upon virus disruption as in the case of preparing PYDV and RTYV for use as coating antigens and in the case of Triton X-100 treatment of PYDV for immunizing rabbits.

During the course of our study aimed at RTYV purification, Inoue and associates independently reported RTYV purification (28). Interestingly, successful purification of RTYV has been accomplished in both laboratories based on the use of Percoll to generate a density gradient upon centrifugation (12,13,28). We found that at concentrations of Percoll lower than 30% (v/v), separation of virus from host materials was not satisfactory while at higher concentrations the virus particles were largely excluded from the gradients. The contaminating Percoll particles in the final purified preparations of virus did not interfere with virion protein analysis in polyacrylamide gel.

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