Reaction of Antiserum Against SDS-Dissociated Rice Dwarf Virus and a Polypeptide of Rice Gall Dwarf Virus

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Accepted for publication 10 May 1985.

ABSTRACT

Matsuoka, M., Minobe, Y., and Omura, T. 1985. Reaction of antiserum against SDS-dissociated rice dwarf virus and a polypeptide of rice gall dwarf virus. Phytopathology 75:1125-1127.

A serological relationship between rice dwarf (RDV) and rice gall dwarf (RGDV) viruses was found with antiserum against virus dissociated with sodium dodecylsulfate (SDS) by the immune blotting technique. The antiserum against SDS-dissociated RDV, which recognized all peptide components of RDV, cross-reacted with one peptide component of RGDV on immune blotting, while antiserum to intact RDV did not react with RGDV. This immune blotting technique with antibody against SDS-dissociated virus could be a highly sensitive method for studying distant serological relationships among viruses.

Rice dwarf (RDV) and rice gall dwarf (RGDV) viruses are both phytoreoviruses in the Reoviridae, but no serological relationship between them has been found. These viruses contain double-stranded RNA divided into 12 segments (2,6). The particle morphology of both viruses is similar, and the molecular weight distributions of their major polypeptides also resemble each other (7,9). These observations suggest that the viruses are related, but antiserum against intact RDV does not cross-react with RGDV in immunodiffusion tests in agar gels (8). An immunodiffusion test with antiserum against intact virus cannot detect all potential differences between the proteins of these two viruses because the antiserum against intact RDV reacts strongly only with the 43,000-dalton (43-kdalton) polypeptide of RDV (5) and antiserum against intact RGDV reacts mainly with 45- and 143-kdalton polypeptides (9).

In the present work, we prepared an antiserum against sodium dodecylsulfate (SDS)-dissociated RDV which reacted with all polypeptides of RDV and also with a polypeptide of RGDV.

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MATERIALS AND METHODS

Virus isolation. RDV and RGDV were purified by the methods of Omura et al (8). The final pellets were resuspended in 0.1 M histidine containing 0.01 M $MgCl_2$, pH 7.0.

Polyacrylamide gel electrophoresis. Electrophoresis in a 10% polyacrylamide gel (slab gel, acrylamide:bisacrylamide = 30:0.8) containing 0.1% SDS was performed by the method of Laemmli (3). Samples were prepared by incubation in 2% SDS and 2% mercaptoethanol at 100 C for 3 min. After electrophoresis, the gels were stained with Coomassie brilliant blue R (CBB). Limited proteolysis in SDS polyacrylamide gels was as described by Cleveland et al (1). After SDS-electrophoresis of RDV or RGDV, the gels were stained with CBB and the stained band of 43-kdalton RDV or 45-kdalton RGDV was excised. The gel slices were put in sample wells of another SDS-containing gel, overlaid with 20 μl of 2 μg/ml *Staphylococcus* V8 protease in 0.125 M tris-HCl, pH 6.8, 0.1% SDS. Digestion was performed in the staking gel for 30 min and was followed by electrophoresis of the products. After the second electrophoresis, the gel was again stained with CBB.

Immunological methods. Antisera against intact particles of RDV and RGDV were prepared in rabbits as before (9). Antiserum against dissociated RDV was produced as follows: Dissociated proteins of RDV were prepared by incubating purified RDV with

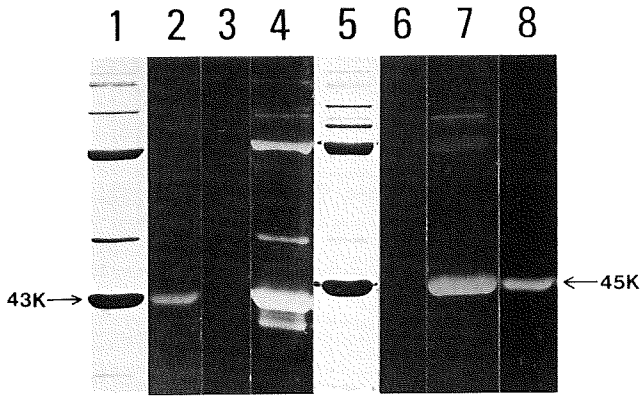

Fig. 1. Immune blotting with antiserum against intact RDV, intact RGDV, and dissociated RDV of RDV and RGDV polypeptides after electrophoresis. Purified and dissociated RDV (lanes 1 to 4) and RGDV (lanes 5 to 8) particles (10 μg per lane) were subjected to SDS-polyacrylamide gel electrophoresis and the gels were stained with Coomassie brilliant blue R (lanes 1 and 5) or analyzed by immune blotting with antiserum prepared against intact RDV (lanes 2 and 6), intact RGDV (lanes 3 and 7), and dissociated RDV (lanes 4 and 8) as described in Materials and Methods. Arrows indicate the positions of the 43-kdalton subunit of RDV and the 45-kdalton subunit of RGDV.

2% SDS and 2% mercaptoethanol at 100 C for 3 min. They were mixed with an equal volume of Freund's complete adjuvant, and the mixture was injected subcutaneously into a rabbit five times at intervals of about 30 days. The rabbit was bled 1 wk after the final injection.

The electrophoretic blotting technique, which was used to characterize immunoreactive protein components of the viruses or immunoreactive peptide-digested fragments, was as described by Matsuoka and Asahi (4). After electrophoresis, the gel was covered with a sheet of nitrocellulose (pore size 0.45 μm; Schleicher & Schuell Co., Keene, NH) which had been wetted with a solution 25 mM tris, 192 mM glycine, and containing 20% methanol (pH 8.3). The polypeptides in the gel were transferred to nitrocellulose paper by electrophoresis at a constant current of 0.7 mA/cm^2 for 1 hr in a Toyo electrophoretic transblot apparatus. The nitrocellulose was then shaken in a saline solution (0.15 M tris-HCl, pH 7.8, 0.9% NaCl) containing 2% bovine serum albumin, shaken in the same saline solution containing 1% antiserum for 1 hr, washed with saline solution, shaken in saline solution containing fluorescein-labeled goat anti-rabbit immunoglobulin G antibody (Cappel Laboratories, Inc., Malvern, PA) for 1 hr and finally washed with saline solution. Serological reactions were detected by fluorescence in long-wavelength range UV-light.

RESULTS AND DISCUSSION

Antiserum against dissociated RDV reacted with all the protein components of RDV (Fig. 1, lane 4) showing that all the protein components were recognized independently as separated antigens when dissociated virus particles were used as immunogens. The antiserum also reacted with the 45-kdalton subunit of RGDV, while antiserum to intact RDV did not react with any polypeptides of RGDV (Fig. 1, lanes 6 and 8). The most likely explanation for this is that the antigenic determinants of intact RDV, "metatopes," according to Van Regenmortel's (11) terminology, are located in the portion of the 43-kdalton polypeptide which composes the surface of the virus particle (5). When dissociated virus was used as an immunogen, other portions of 43-kdalton polypeptide, which are not recognized by antiserum to intact RDV, also become antigenic determinants. These hidden antigenic determinants are termed "cryptotopes" (11). These results suggest that the related antigenic determinants of RDV and RGDV are located only on the portions of the 43- and 45-kdalton polypeptides which are not antigenic in the intact virion. Dissociated capsid proteins of bromoviruses also are more closely related than intact virions (10).

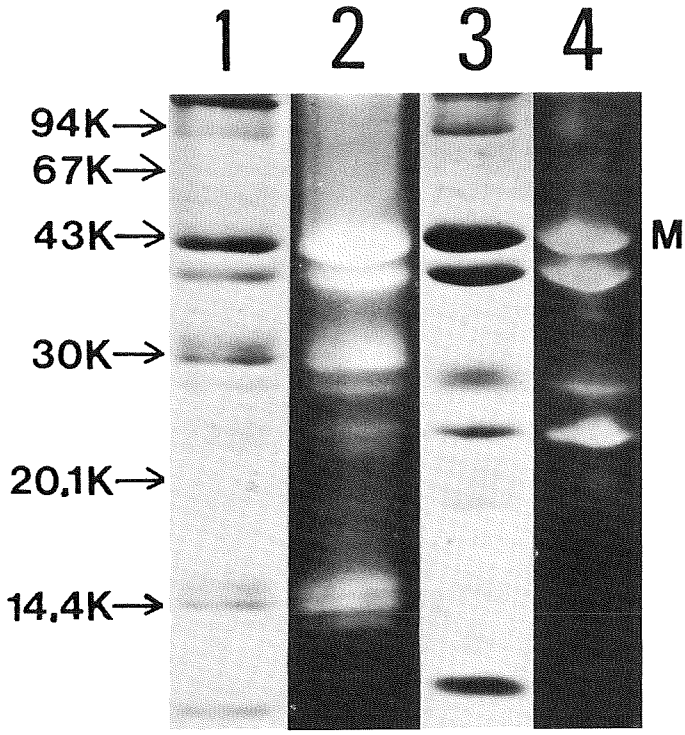

Fig. 2. Peptide maps of the 43-kdalton subunit of RDV and the 45-kdalton subunit of RGDV. After SDS-polyacrylamide gel electrophoresis of RDV or RGDV (Fig. 1, lanes 1 and 5), the bands containing the 43-kdalton and the 45-kdalton protein were cut from the gels and subjected again to SDS-polyacrylamide gel electrophoresis after addition of 20 μl of 2 μg/ml *Staphylococcus* V8 protease. This gel was stained with Coomassie brilliant blue R (lanes 1 and 3) or subjected to immune blotting with antiserum against dissociated RDV (lanes 2 and 4). Lanes 1 and 2 contained about 10 μg of 43-kdalton subunit of RDV and lanes 3 and 4 a similar quantity of 45-kdalton subunit of RGDV. Arrows indicate the positions of the molecular weight markers: phosphorylase b (94 kdaltons), albumin (67 kdaltons), ovalbumin (43 kdaltons), carbonic anhydrase (30 kdaltons), trypsin inhibitor (20.1 kdaltons), and lactalbumin (14.4 kdaltons). M indicates the position of the undigested 45-kdalton subunit of RGDV.

If this explanation is correct, only portions of the 45-kdalton subunit of RGDV react with dissociated RDV antiserum. Therefore, we investigated the immunological reactivities of peptide fragments produced from either the 43-kdalton subunit of RDV or the 45-kdalton subunit of RGDV by digestion with *Staphylococcus* V8 protease against antiserum to dissociated RDV in immune blotting. The antiserum reacted with almost all fragments produced from the 43-kdalton subunit of RDV (Fig. 2, lane 2); the antiserum reacted strongly with three fragments, 39.5, 28, and 24 kdalton from the 45-kdalton subunit of RGDV, but not with the 29-, 19-, and 12-kdalton fragments (Fig. 2, lane 4). These results support the idea that some cryptotopes of the 43-kdalton subunit of RDV are closely related to those of the 45-kdalton subunit of RGDV.

No serological relationship has been demonstrated between RDV and RGDV, because antiserum against intact virions did not cross react (8). However, we have shown that a serological relationship exists between the two viruses when an antiserum prepared against dissociated RDV particles was used. It appears that the immune blotting technique used with antiserum against SDS-dissociated virus is useful for detecting common antigenic determinants hidden in the virus particle and may help to detect group-specific antigens of remotely related viruses.

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