Use of Sodium Dodecyl Sulfate in Serodiagnosis of Barley Stripe Mosaic Virus in Embryos and Leaves

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ABSTRACT

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Sodium dodecyl sulfate (SDS) was used in the serodiagnosis of barley stripe mosaic virus (BSMV) in embryos and leaves. Antisera elicited by SDS-treated preparations of partially purified BSMV were produced in rabbits and then used in an agar gel immunodiffusion medium amended with SDS. The serodiagnostic test was based on a modified Ouchterlony double-diffusion technique in which SDS replaced Leonil SA as the viral degrading agent. Filter paper disks served as seroreactant depots. The SDS-disk test worked equally well with freshly prepared or lyophilized seroreactants. More than 50,000 embryos were assayed successfully. The procedure shows promise for field surveys and seed testing worldwide.

To detect seedborne barley stripe mosaic virus (BSMV) in barley, the embryo test developed by Hamilton (6) was used routinely by the Montana Seed Laboratory for the Montana Seed Growers Association from 1966 to 1976. This adaptation of the Ouchterlony double-diffusion method in agar gel included two important earlier techniques employed by Hamilton: (i) use of agar amended with Leonil SA (LSA) detergent (8) and (ii) use of lyophilized antisera and antigens in paper disks (7). The anionic detergent improved the diffusibility of the viral antigens and thus enhanced the sensitivity of the test. The lyophilized seroreactants were convenient for field surveys and seed testing.

In 1976, the embryo test was modified because suitable antisera were difficult to produce, LSA detergent was no longer commercially available, and unidentified, nonviral precipitates formed when embryos of certain barley cultivars were tested, making results difficult to interpret. The few suitable antisera produced for the embryo test required large amounts of inject immunogen irrespective of immunogen form or route of injection. Whole virus and pyridine (12) or pyrrolidine (13) degraded virus appeared to possess about equal immunogenicity. This observation agreed with the results of Atabekov and colleagues (1,3) who reported that 100–150 mg of purified BSMV or its protein had to be administered by intramuscular or intravenous injections, or both, to each rabbit to elicit an analytical antiserum.

The modified embryo test currently used by the seed laboratory differs from the one developed by Hamilton (6) in that (i) the antisera are elicited by sodium dodecyl sulfate (SDS)-treated preparations of partially purified BSMV and (ii) the agar gel immunodiffusion medium is amended with SDS.

This article describes the development of this modified procedure for serodiagnosis of barley stripe mosaic virus (BSMV) facilitated by SDS in embryos and leaves. It also describes the production, evaluation, and manipulation of the antisera elicited by SDS-treated antigens of BSMV.

MATERIALS AND METHODS

Virus. The MI-3 strain of BSMV provided by R. F. Eslick was used. The virus was propagated in Vantage barley plants (*Hordeum vulgare* L.) in a greenhouse. About 50 seeds from an infected seed lot of Vantage barley were sown per flat. This seed lot produced more than 70% infected progeny. Plants infected via seed transmission were used for the sake of convenience since no mechanical inoculation was required to establish an infection.

Preparation of partially purified virus. Leaves of seedlings at the three to five leaf stage were processed according to the purification schedule of Batchelor (4). Briefly, 300 g of tissue were clarified by homogenization with a blender in 300 ml of 0.05 M sodium borate buffer, pH 8.2, which contained 0.1% of 2-mercaptoethanol (2-ME), 150 ml of *n*-butanol, and 150 ml of chloroform. Afterwards the preparation was centrifuged, precipitated with 8% polyethylene glycol 6000, and partially purified by 2 cycles of differential centrifugation. Final pellets were resuspended in 0.01 M sodium borate buffer and stored at 4 C. Average virus yield from 12 preparations was 145.2 mg/kg of leaf tissue (Range: 322.0 mg—42.5 mg/kg). The 260:280 ratio for the 12 preparations averaged 1.86. This unusually high ratio for BSMV suggested the presence of contaminating host nucleic acid.

Preparation of immunogen. The procedure of Purcifull and Batchelor (10) was used to prepare inject immunogen. Usually 2 ml of virus (10 mg/ml) were combined with SDS and 2-ME to give a final concentration of 3% (w/v and v/v, respectively). This was followed by heat denaturation, centrifugation, Sephadex liquid column chromatographic separation, and Sephadex solute concentration of the denatured viral protein. This procedure generally yielded 3 ml of immunogen solution containing about 12 mg of viral protein.

Immunization and collection of antisera. Emulsification, injection of the immunogen, and antisera collection procedures were similar to those reported by Purcifull and Batchelor (10). Injections of 1 ml of immunogen containing 1–4 mg of denatured virus, emulsified 1:1 with adjuvant, was administered intramuscularly into each of 11 New Zealand white rabbits. Not less than seven injections were given to each rabbit. The collection of antisera continued during a 1–6 mo period.

SDS-disk test. The SDS-immunodiffusion procedure based on the Ouchterlony technique was assessed for the detection of BSMV in embryos and leaves. In this modification of Hamilton's method (6), SDS replaced LSA. Filter paper disks (6-mm diameter) served as seven- or five-place circular patterns, with peripheral disks 4 mm from a central antibody containing disk. The gel medium was 0.6% Noble agar, 0.1% SDS, and 0.1% sodium azide (all w/v) in 0.05 M sodium borate buffer, pH 8.0. About 4.0 ml of medium was dispensed per 9-cm petri plate.

Antigen manipulation. Extracts of embryos were prepared freshly as described by Hamilton (6). Paper disks containing the

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embryo extracts were transferred directly to serological plates. Most embryos were from seed of Vantage barley. Some seeds were infected with the MI-3 strain of BSMV. Other embryos came from barley cultivars that had been shown to cause formation of nonviral precipitates when tested by Hamilton's LSA procedure (6).

Leaf extracts were obtained from pressed or triturated leaves of Vantage, Atlas, and Blackhulless barley infected with either the MI-3 strain or other strains of BSMV. Uninfected leaves of these barleys were processed similarly.

Extracts from pressed leaves were made by squeezing one leaf at a time between a folded piece of plastic-coated paper placed in a vise-grip pliers with modified jaws. Each undiluted extract was used to soak one or more paper disks. Some of these extracts were used immediately after preparation or were stored at 4 C, and others were lyophilized in paper disks. Ten disks were soaked in 0.1 ml of extract following the procedure of Hamilton (7). In addition, the same lyophilization treatment was given to control disks that were soaked in a 1% SDS solution in distilled water. The lyophilized leaf extracts were kept in the refrigerator until needed. The lyophilized disks were applied directly to the serological plates and required no wetting agent.

Extracts from triturated leaves were prepared in distilled water (1 g/ml) using a mortar and pestle. These extracts were used immediately after preparation or were kept at 4 C. Others were lyophilized in a manner similar to the one described by Purcifull et al (11). Generally, 2–5 ml of extract were freeze-dried in a serum bottle. Prior to lyophilization, 1.0% SDS (w/v) was added to a few extracts. The lyophilized extracts were also stored in the refrigerator until needed. Lyophilized leaf extracts were reconstituted to either original or partial volumes in distilled water. The extracts from triturated leaves were used for wetting disks for the SDS-disk test.

Partially purified preparations of BSMV, MI-3 strain, were diluted in distilled water, and kept cold in the refrigerator or stored frozen until use. Thawed and cold virus suspensions were applied to the disks for assessment of the SDS-disk test.

Antisera manipulation and evaluation. Antisera and normal sera were stored either by freezing or by lyophilization until use. Sera were lyophilized in serum bottles and in paper disks (0.1 ml of serum/10 disks) according to the procedure of Hamilton (7). The freeze-dried sera in bottles were reconstituted to original or partial volumes in nonsterilized distilled water just before use.

Frozen, lyophilized-reconstituted antisera, and lyophilized antisera in disks were treated with fresh embryos and leaf extracts, lyophilized leaf extracts, and partially purified BSMV.

Titer determinations for the potency evaluations were made by testing serially diluted antisera for precipitating activity against SDS-treated preparations of partially purified BSMV. Liquid antisera were diluted directly in one of the three diluents, but lyophilized sera were first reconstituted in distilled water, followed by dilution in one of the diluents. The diluents were: (i) phosphate buffered saline (0.01 M potassium phosphate buffer, pH 7.0, and 0.85% sodium chloride [PBS]), (ii) 1.0% bovine serum albumin in distilled water (BSA), or (iii) normal serum (nonimmune serum [NS]). The serological application of each of these diluents has been shown previously (10).

To assay the antisera for sensitivity to different concentrations of viral antigen, serial dilutions of partially purified BSMV in distilled water were tested for precipitating activity against undiluted antisera. Viral concentrations were estimated by spectrophotometry ($E^{0.1\%}_{260nm} = 2.6$).

The specificity of the antisera was determined against fresh and lyophilized leaf extracts and concentrated host protein from leaves of uninfected barley plants. The concentrated host protein was prepared following the early steps of the procedure described for the preparation of partially purified BSMV, and it consisted of the resuspended pellets obtained from the first high-speed ultracentrifugation (78,000 *g* for 90 min).

Cross-reactivity of the MI-3 strain of BSMV was assessed using SDS-antisera to MI-3 against infected leaf sap containing other strains of the virus. Those others included strains known to possess 2, 3, or 4 RNA components (provided by L. C. Lane, University of Nebraska) and uncharacterized strains obtained from North Africa and the Middle East. Petri plates were observed at 24, 48, and 72 hours for the presence and prominence of immunospecific precipitation lines.

RESULTS

The SDS-disk test using BSMV-infected embryos revealed immunospecific precipitates (Fig. 1). The use of BSMV infected leaves also resulted in specific antigen-antibody precipitates. Liquid and both kinds of lyophilized antisera produced immunoprecipitates of equal strength, but precipitin lines between infected embryos and antisera disks were more prominent than the lines between infected leaves and antisera. This was true for either homologous or heterologous antigen-antibody combinations. Antisera concentrated by lyophilization followed by reconstitution to partial volume developed immunospecific precipitates about as strong as the unconcentrated antisera. Nonviral precipitates were absent when antisera impregnated disks were allowed to react against either healthy embryo extracts or leaves. No immunospecific precipitates formed between embryo or leaf extracts and liquid or lyophilized normal sera disks. Nonviral reactions caused presumably by sera-SDS interactions were visible as narrow precipitation rings at the circumference of some but not all antisera and normal sera disks. No problem arose, however, in distinguishing them from specific reactions due to their position at the sera depots.

Good diagnostic antisera were produced by rabbits given 50–70 mg of total immunogen per rabbit. Undiluted antisera in either a liquid or a lyophilized-reconstituted state produced prominent immunoprecipitation lines against 100 mg/ml of BSMV. The antisera diluted in normal sera, the most effective type of diluent in enhancing titers, frequently gave titers of 1:4 against the 100 mg/ml BSMV. BSA was a slightly more effective diluent than PBS for increasing antisera titers.

Sensitivity determinations of some unconcentrated antisera indicated that partially purified BSMV antigen could be detected at antigen concentrations of 10–25 μg/ml.

When selectivity experiments were conducted with the antisera against leaf extracts and partially purified BSMV, immunospecific precipitates formed only with viral antigens. No similar precipitates were seen when antisera were allowed to react with healthy leaf extracts. Liquid and lyophilized antisera and fresh and lyophilized-reconstituted leaf extracts produced lines of about equal intensity. Moreover, concentrated barley host proteins did not form precipitates with the variously treated antisera.

Results of the cross-reactivity tests showed that the antisera

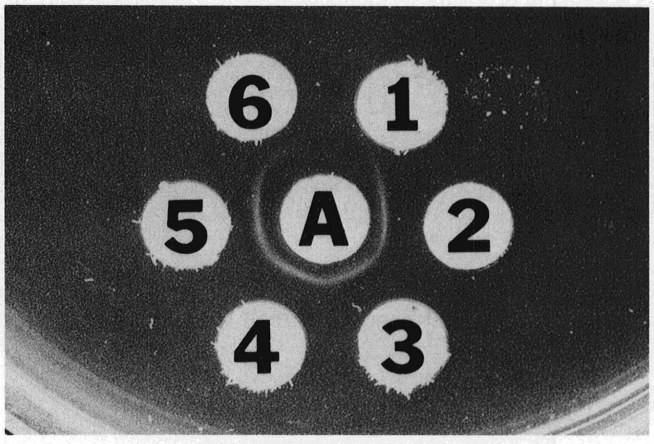

Fig. 1. Sodium dodecyl sulfate (SDS)-disk test. Serodiagnosis of barley stripe mosaic virus in embryos with an antiserum produced to the MI-3 strain of the virus. The embryos were freshly crushed and the antiserum had been frozen and was thawed just before use. Disk A = undiluted antiserum; disks 2, 3, 4, and 5 = embryos infected with the MI-3 strain of BSMV; disks 1, and 6 = healthy embryos.

elicited by the MI-3 strain of BSMV were diagnostic for all other strains of the virus tested. Using infected leaf extracts and MI-3 derived antisera, all strains reacted in an homologous manner. The MI-3, MI-1, and MI-2 strains were previously shown to have three RNA components (M. K. Brakke, *personal communication*), and when all were assayed against several MI-3 antisera, the immunospecific precipitate lines were fused.

Type (ATCC 69) and Canadian severe strains that presumably have two RNA components, the Norwich strain that presumably has three RNA components, and the Argentina mild strain that has four components (9) also appeared to be serologically identical to MI-3. Because only antisera to the MI-3 BSMV strain were prepared for this study, any serologically deduced homologous relationship between strains can be interpreted only as very tentative. For all practical purposes, however, antisera derived from MI-3 BSMV can be used for serological diagnosis in an agar gel immunodiffusion system containing SDS, regardless of the strain present. This conclusion is particularly important when considering the development of an antiserum to be used in any program of mass testing of barley as is presently being conducted at Montana State University.

DISCUSSION

Serodiagnosis of BSMV in embryos and leaves can be accomplished successfully with SDS-treated BSMV and its homologous antisera. Sodium dodecyl sulfate is not only a suitable substitute for LSA as a detergent to chemically disrupt the virus and make is diffusible in an agar system, but it is also an effective viral degrading agent for the preparation of useful soluble immunogen. Presumably in the presence of 1.0% SDS, the BSMV capsid dissociates into monomeric protein subunits of about 21.5×10^3 daltons (2).

When proper amounts of inject immunogen are administered, good diagnostic antisera can be developed. As undiluted, unfractionated antisera they possess high titers. Our results suggest that 10 mg/ml per rabbit should be given for each injection. The first injection should be succeeded 1 mo later by the second injection. The third and subsequent two to four injections should be at 2-wk intervals, resulting in 50–70 mg of total immunogen per rabbit.

The decision to develop and apply the SDS procedure for the serodiagnosis of BSMV in Montana in preference to using some other serological technique was based on the following reasons: (i) published serological data indicate that the SDS test would provide about the same sensitivity as but greater reliability than the LSA assay, (ii) the SDS test would substitute easily for LSA immunodiffusion method, (iii) the SDS procedure could be instituted readily with existing facilities, and (iv) laboratory personnel would require minimal training to learn the SDS procedure because the SDS immunodiffusion technique is similar to the LSA method.

Results of serological testing in Montana during 1976 and 1977 support our decision regarding the SDS procedure. The SDS antisera were used successfully in tests by the Montana Seed Testing Laboratory for the Montana Seed Growers Association and by the Plant Virology Laboratory. The sensitivity of the SDS test was equal to or greater than that of the LSA assay. Furthermore, the SDS test was more reliable than the LSA assay because the spurious precipitate was eliminated.

During 1976 and 1977, by combining the existing method for excising embryos (5) with the SDS-disk test, the seed laboratory assayed 59,800 embryos (299 seed lots, 200 embryos per lot) for seedborne BSMV and determined that 37 lots (12.4%) were infected. Twenty-six lots (8.7%) were more than 5% infected. By comparison, LSA assays of the same number of embryos in 1975 detected virus in 38 (12.7%) seed lots, 11 (4.0%) of which had levels of infection greater than 5 percent.

The only nonviral reaction observed with the SDS test was the serum-SDS interaction (10), which is identifiable and does not interfere with serological interpretation. No serum-plant extract interaction occurred, even when concentrated host proteins were allowed to react against SDS antisera.

Lyophilized seroreactants show great promise for field surveys and seed testing not only in Montana, but also in developing countries where they can be used by workers after they have received minimal training. Freeze-dried antisera, normal sera, and SDS-treated antigens can be shipped worldwide without concern for refrigeration or viral infectivity.

Detection of BSMV in leaves also was accomplished with the SDS-well test, which was similar to the SDS-disk test. For the well test, seroreactant depots were 5-mm wells arranged in a seven-place circular pattern with the peripheral wells 5 mm from the central well. The immunodiffusion medium contained 0.6% Noble agar, 0.5% SDS, and 1.0% sodium azide (all w/v) in deionized water. Ten to 12 ml of medium were added per 9-cm plate.

LITERATURE CITED

1. ATABEKOV, J. G., and V. K. NOVIKOV. 1966. Some properties of the nucleoprotein of barley stripe mosaic virus and of its structural components. Biokhimia 31:134-142. (English translation).
2. ATABEKOV, J. G., V. K. NOVIKOV, N. A. KISELEV, A. S. KAFTANOVA, and A. M. EGOROV. 1968. Stable intermediate aggregates formed by the polymerization of barley stripe mosaic virus protein. Virology 36:620-638.
3. ATABEKOV, J. G., N. D. SCHASKOLSKAYA, S. P. DEMENTYEVA, G. N. SACHAROVSKAYA, and E. P. SENCHENKOV. 1968. Serological study on barley stripe mosaic virus protein polymerization. I. Immunodiffusion, immunoelectrophoretic characteristics, and absorption experiments. Virology 36:587-600.
4. BATCHELOR, D. L. 1974. Immunogenicity of sodium dodecyl sulfate-denatured plant viruses and plant viral inclusions. Ph.D. dissertation, University of Florida. 81 p.
5. DAVIS, D. J., and R. WALLACE. 1967. Serodiagnosis for barley stripe mosaic virus in Montana. Proc. Assoc. Off. Seed. Anal. 57:132-137.
6. HAMILTON, R. I. 1965. An embryo test for detecting seed-borne barley stripe mosaic virus in barley. Phytopathology 55:798-799.
7. HAMILTON, R. I. 1965. Lyophilized antiserum in paper disks for serodiagnosis of barley stripe mosaic. Virology 26:153-154.
8. HAMILTON, R. I. 1964. Serodiagnosis of barley stripe mosaic facilitated by detergent. Phytopathology 54:1290-1291.
9. LANE, L. C. 1974. The components of barley stripe mosaic and related viruses. Virology 58:323-333.
10. PURCIFULL, D. E., and D. L. BATCHELOR. 1977. Immunodiffusion tests with sodium dodecyl sulfate (SDS)-treated plant viruses and plant viral inclusions. Fla. Agric. Exp. Stn. Bull. No. 788. 39 p.
11. PURCIFULL, D. E., S. R. CHRISTIE, and D. L. BATCHELOR. 1975. Preservation of plant virus antigens by freeze-drying. Phytopathology 65:1202-1205.
12. SHEPARD, J. F. 1972. Gel-diffusion methods for the serological detection of potato viruses X, S and M. Mont. Agric. Exp. Stn. Bull. No. 662. 72 p.
13. SHEPARD, J. F., G. A. SECOR, and D. E. PURCIFULL. 1974. Immunochemical cross-reactivity between the dissociated capsid proteins of PVY group plant viruses. Virology 58:464-475.