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PURIFICATION OF SOYBEAN MOSAIC VIRUS

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Symptoms of soybean mosaic virus (SMV) in soybean include mosaic, rolling and puckering of foliage, and chlorosis of older leaves. The virus may significantly reduce yields of soybeans and cause a mottling of the seed (1). The characterstic particles associated with SMV are long flexuous rods about 740 mm in length. Galvez (2) reported aggregation of the particles during purification which prevented isolation of the virus sufficiently pure to produce antiserum free of antibodies to normal host antigens. Ross (4) reported purificaton of SMV and production of antisera free from host antibodies by using 0.5 M citrate buffer during low speed centrifugation and suspending the pelleted virus after high speed centrifugation in a weak borate buffer. However, this procedure did not eliminate virus loss due to aggregation of particles during the high speed centrifugation.

Several viruses, including those with particles that are long flexuous rods, have been purified by filtration through granulated agar columns (3, 5, 6, 7, 8). The object of this investigation was to purify SMV without aggregation by using agar gel filtration combined with alternate high and low speed centrifugation.

MATERIAL AND METHODS

The virus used was a strain of SMV used in past host-range studies (9). Soybean (*Glycine max* var. Hill) was used for increasing the virus and as a systemic indicator host. Inoculations were made by rubbing carborundum-dusted leaves with gauze pads either soaked in sap produced by grinding infected soybean leaves on 0.5 M potassium phosphate buffer (pH6.5) or soaked in virus preparations obtained during the course of purification.

Low-speed centrifugations were performed in a Lourdes LCA-1 centrifuge at 4°C. The virus was pelleted at 30,000 rpm for 60 min. in the No. 30 rotor of a Spinco model L ultracentrifuge.

In this study a column similar to that described by Van Regenmortel (7) was used. Four percent lonagar No. 2 was prepared and ground in a Waring Blender until the granules could pass through a 40-mesh sieve but not a 60-mesh sieve. The granules

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were packed in a glass column 60 cm by 2 cm, covered with a thin pad of glass wool, and washed with a liter of 0.01 M sodium borate buffer (pH8.3). Ths buffer was used throughout purification unless otherwise noted. Column experiments were conducted at about 22°C.

Dialysis was performed in 4 liters of 50% polyethylene glycol with an Oxoid multiple dialyzer at 4°C.

Ultraviolet absorption spectra were taken with a Beckman DB-G spectrophotometer with virus diluted sufficiently to place peaks within an absorbance range of 0.2-0.8.

Virus antiserum was produced by injecting a rabbit intramuscularly with 1 mg of purified virus in 1 ml of buffer emulsified with 1 ml of Freund's incomplete adjuvant (Difco), with 3 injections given at 6-day intervals. Blood samples of 50 ml were collected on each of 2 days at a 7-day interval starting 7 days after the last injection. Antiserum dilution end points were determined by microprecipitin tests. Results were recorded after the tests had been incubated 1 hour at 37°C and left overnight at 4°C.

Partial purification of the virus followed closely the technique used by Ross (4). Harvested SMV-infected soybean leaves (100 g) were homogenized in 200 ml of 0.5 M sodium citrate with 1% 2-mercaptoethanol. The pulp was extracted by straining the extract through 2 layers of cheese cloth. While the extract was stirred, 16 ml of n-butanol was slowly added. After curdling was evident, the preparation was placed in a cold room overnight at 4°C. The extract was then clarified at 10,000 rpm for 10 minutes in the 9RA rotor of the Lourdes centrifuge. The supernatant was decanted through glass wool and an amber liquid was obtained. The volume of the liquid was reduced to 25 ml by dialysis against 50% polyethylene glycol. To reduce loss of virus adhering to the tubing during dialysis, 50 ml of the liquid in each of 4 tubes was reduced to 25 ml, the contents of the 4 tubes combined into 2 tubes, and process repeated until 25 ml remained in one tube. Following concentration sufficient sucrose was added to increase density of the liquid 10% by weight and the liquid placed on the column under 70 ml of buffer. The liquid was filtered through the column at the rate of 20 ml/hour. The effluent passed through an ISCO ultraviolet analyzer with wave length set at 254 mu and was collected in 6 ml volumes by a fraction collector.

RESULTS AND DISCUSSION

Infectivity was associated with the initial peak on the UV analyzer graph indicating that the virus was eluted between 50 and 120 ml and followed by plant components. Considerable overlapping of the two fractions was evident.

Fractions containing the 50 to 100 ml portion of the elution volume were combined and the virus pelleted by high speed centrifugation. The pellets were resuspended in 2 ml borate buffer. Absorption spectra of fractions taken in the 50 to 70 ml range of the elution volume showed the maxium peak at 257 mu and a minimum at 234 mu.

Virus antiserum tested against the purified virus preparation and against clarified sap from SMV-infected plants gave the flocculent type of precipitate characteristic of rod shaped viruses. Some precipitation was observed with clarified healthy plant sap. The antiserum dilution-end-point against the purified virus was 1:16. The purification process was carried out several times. Dry weight determinations indicated that approximately 2.1 mg vrus were obtained per 100 g plant tissue.

Separation of SMV and host protein using the agar column was difficult due to overlap of the two components during filtration. High speed centrifugation was found to be the most efficient method of concentrating the purified virus, although this procedure resulted in partial loss of infectivity. A sharp peak was obtained at both the maximum of 257 mu and minimum of 234 mu on the spectrophotometer. Ross (4) reported a broad maximum peak at 260-265 mu and a sharp minimum peak at 245 mu, along with a tryptophan shoulder at 290 mu, which was not evident in the present studies.

The overlapping of the virus and plant protein during filtration through the column explains the presence of precipitate when clarified healthy plant sap was reacted with the virus antisera.

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