Purification of Soybean Mosaic Virus by Affinity Chromatography Using Monoclonal Antibodies

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SUMMARY

Immunoaffinity columns were prepared by covalently binding monoclonal antibodies specific for soybean mosaic virus (SMV) to an agarose support matrix. SMV, purified by binding and elution, contained no contaminating proteins detectable by SDS–polyacrylamide gel electrophoresis. Affinity-purified SMV was essentially identical to virus purified by standard procedures in its infectivity, u.v. absorbance spectrum ratio of $A_{280}/A_{260}$, sedimentation coefficient, electrophoretic pattern of coat protein, morphology and antigenicity. This method of purification is rapid, reproducible, and yields highly purified virus preparations. It may therefore be applicable in the purification of a wide variety of plant viruses.

INTRODUCTION

Purification of plant viruses is often difficult and time-consuming. Many purification procedures involve extraction, clarification, precipitation, differential centrifugation, density gradient centrifugation and concentration to achieve highly purified virus preparations (Matthews, 1981). Gel filtration procedures are generally less time-consuming but require much processing of tissues before chromatography and often yield preparations contaminated with high molecular weight host components (Hewish & Shukla, 1983).

Antibodies immobilized onto support matrices have been used to immunopurify a wide variety of biological compounds such as bacterial proteins (Sjoberg & Holmgren, 1973), enzymes (Melchers & Messer, 1970; Erickson & Steers, 1970), hormones (Akanuma et al., 1970; Weintraub, 1970; Murphy et al., 1973), viral proteins (Oroszlan et al., 1975) and animal viruses (Kenyon et al., 1973; Sweet et al., 1974). Monoclonal antibodies (MAbs) are attractive reagents for use in immunoaffinity procedures (Secher & Burke, 1980; Novick et al., 1982, 1983) because of their high specificity (Köhler & Milstein, 1975), and because of the high titres which can be obtained.

We report purification of soybean mosaic virus (SMV) by affinity chromatography using an immunosorbent prepared by covalently coupling MAbs to agarose. The SMV affinity-purified from plant sap was essentially identical to virus purified by standard methods, suggesting the potential for use of the technique in large-scale purification of a variety of plant viruses.

METHODS

Virus and antisera. SMV (Ia 75-16-1) was purified from infected Glycine max (L.) Merr. cv. Williams as described by Hill & Benner (1980). Virus concentration was estimated spectrophotometrically by using $A_{660}^\text{nm} = 2.4$ (Purcifull, 1966). The MAbs S1 and S2 used in this study are described elsewhere (Hill et al., 1984).

Purification of IgG and preparation of immunoaffinity column. MAbs were affinity-purified from ascites fluid by using Protein A–Sepharose CL-4B (Pharmacia). IgG was bound from solution in 0.1 M-NaHPO$_4$ pH 8.0, and eluted with 5% acetic acid in saline, adjusted to pH 3.0 with 0.1 M-HCl. Concentrations of IgG were determined using $A_{280}^\text{nm} = 1.4$ after adjustment of samples to between pH 7.0 and 8.0 with 1 M-NaOH.

Approximately 8.5 mg Protein A-purified S2 MAb was dialysed against three changes of 1 l of 0.05 M-HEPES pH 8.0. The IgG was added to an equal volume (5.3 ml) of Affi-Gel 10 beads (Bio-Rad, Richmond, Ca., U.S.A.),
which had been washed by centrifugation immediately before addition of the IgG twice with 30 ml ice-cold deionized distilled water (DD-H2O) and once with 30 ml 0.05 M-HEPES pH 8.0 at 4 °C. The mixture was rotated overnight on an end-over-end mixer at 4 °C to allow coupling of the immunoglobulins. By spectrophotometry, approximately 7-6 mg of S2 IgG was found to be bound to the affinity matrix (90% coupling efficiency). The immunoadsorbent was placed in a 9 × 150 mm chromatographic column (Pharmacia) and was washed with 100 ml 0.05 M-sodium borate pH 8.0 containing 0.15 M-NaCl (BBS). To prevent further non-specific adsorption of proteins to the affinity matrix, 4 ml of a 5 mg/ml solution of bovine serum albumin in BBS was added, and the column was washed with BBS until the eluate had an A230 of less than 0.05. The column was then washed with 50 ml 0.05 M-sodium borate pH 8.0 (BB), 50 ml DD-H2O, adjusted to pH 3.0 with 0.1 M-HCl, and 50 ml of BBS before the first addition of virus.

**Affinity purification of SMV.** For optimization and analysis of the affinity chromatographic procedure, purified SMV diluted in BB was layered on the column and incubated overnight at room temperature with the affinity matrix. To remove unbound virus, the column was washed with BB until the effluent A250 was less than 0.05. The column was then washed with 25 ml DD-H2O, adjusted to pH 8.0 with 0.1 M-NaOH, and bound virus was eluted with DD-H2O pH 3.0. Fractions containing virus were mixed with 0.1 vol. 0.01 M-sodium phosphate pH 7.0, and the virus concentration was determined by spectrophotometry. For purification of SMV from plant sap, virus was diluted in plant extract obtained by triturating 5 g of leaves from uninfected soybean, cv. Williams, in 10 ml 0.05 M-sodium-potassium phosphate pH 7.2, or alternatively 25 g of soybean leaves were triturated with 40 ml of buffer 3 weeks after inoculation. Before use, the extract was expressed through three layers of cheesecloth and centrifuged for 1.5 min at 8740 g in a Beckman microfuge B. After addition of plant extract to the column, the extract was incubated with the affinity matrix overnight at room temperature and eluted as previously described.

**Characterization of affinity-purified SMV.** Several characteristics of affinity-purified virus were compared with those of virus purified by standard procedures (Hill & Benner, 1980) and with ‘mock-purified’ virus. ‘Mock-purified’ virus was prepared by diluting purified virus with at least 30 vol. DD-H2O pH 3.0, incubating it for 5 min at room temperature, and mixing the virus solution with 0.25 vol. 0.05 M-sodium phosphate pH 7.0. The virus preparations were concentrated by ultracentrifugation at 65000 g for 2 h and resuspended in 0.05 M-sodium phosphate pH 7.0.

**Infectivity.** The infectivity of the preparations was estimated by using a detached half-leaf local lesion assay (Milbrath & Soong, 1976).

**Gel electrophoresis.** SDS-slab gel electrophoresis was performed using 12% and 5% polyacrylamide resolving and stacking gels, respectively (Laemmli, 1970). Protein was prepared by heating virus preparations with an equal volume of 0.125 M-Tris–HCl, 2% SDS, 2% 2-mercaptoethanol, 30% (v/v) glycerol for 5 min at 100 °C. Protein molecular weights were determined by the method of Shapiro et al. (1967), using phosphorylase B (92500), bovine serum albumin (66200), ovalbumin (45000), carbonic anhydrase (31000), soybean trypsin inhibitor (21500) and lysozyme (14400) as molecular weight standards.

**Sucrose gradient centrifugation.** Sedimentation coefficients were determined as described by Brakke & Van Pelt (1970) using purified tobacco mosaic virus and cowpea mosaic virus, strain Sb, as standards. Linear–log sucrose gradients, prepared as described (Jackson et al., 1977) for an SW41 rotor (Beckman) at 6 °C using a particle buoyant density of 1.3, were run at 77100 g for 2.5 h.

**Electron microscopy.** The morphology of affinity-purified preparations was examined by negatively staining particles with 1% aqueous uranyl acetate.

**ELISA.** Antigenicities of the viral preparations were compared by using a monoclonal antibody-based ELISA. Dilutions of virus in 0.05 M-sodium phosphate pH 7.0 were incubated overnight at 4 °C in plates (Immulon I, Dynatech, Alexandria, Va., U.S.A.) coated with 275 ng/well of MAb S2 and blocked by addition of 0.01 M-phosphate-buffered saline (PBS) pH 7.4 containing 1% bovine serum albumin and 0.05% Tween 20. Biotinylated MAb S1 (Bayer et al., 1979) was added to wells (60 ng/well) and incubated for 1 h at 37 °C followed by addition of 12.5 ng/well of alkaline phosphatase-conjugated avidin (Sigma) for 1 h at 37 °C, and 50 ng/well of the substrate p-nitrophenyl phosphate in 10% diethanolamine pH 9.8 (Voller et al., 1979). Four washes with PBS containing 0.05% Tween 20 preceded each addition. The substrate was allowed to incubate 30 min at 37 °C, at which time 50 µl/well of 3 M-NaOH was added, and the absorbance at 410 nm was determined using a Dynatech Minireader II.

**RESULTS**

Affinity-purified SMV was compared with virus purified by standard procedures and with ‘mock-purified’ virus, which had been incubated in DD-H2O pH 3.0 after purification. Tests showed that the infectivity of SMV was not significantly affected either by incubation at pH 3.0 or by affinity chromatography. In some experiments, affinity-purified preparations gave higher or lower numbers of local lesions than untreated or ‘mock-purified’ controls but, in all cases, the virus was clearly infectious (Table 1). This variation was probably due to fluctuations in the
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Fig. 1. Absorbance profile of (a) untreated virus, (b) ‘mock-purified’ virus and (c) affinity-purified SMV. All three preparations were adjusted to approximately equal A260 values.

Table 1. Comparison of the infectivities of untreated, ‘mock-purified’ and affinity-purified SMV

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Untreated SMV</th>
<th>‘Mock-purified’ SMV</th>
<th>Affinity-purified SMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>33</td>
<td>From stock†</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td></td>
<td>From sap</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>16</td>
<td>30</td>
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<td></td>
<td>33</td>
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<tr>
<td>3</td>
<td>22</td>
<td>51‡</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>56‡</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>41</td>
<td>105§</td>
<td></td>
</tr>
</tbody>
</table>

* Data are the total numbers of local lesions per eight (no. 1 and 2) or ten (no. 3, 4 and 5) half-leaves from leaves of Phaseolus vulgaris L. cv. Top Crop. Virus concentrations were adjusted to be equal concentrations (A260 = 0·5 for experiments 1 and 2; A260 = 0·75 for experiments 3, 4 and 5). Data on a horizontal row are for the inocula compared on opposite half-leaves.

† Stock virus purified as described by Hill & Benner (1980).
‡ Stock virus mixed with triturated uninfected Williams soybean leaf extracts.
§ Virus purified from extract of Williams soybean leaves prepared 3 weeks after inoculation.

sensitivity of the biological assay rather than to intrinsic differences in infectivity because we have been unable to achieve a statistically significant relationship between virus concentration and number of local lesions using this assay (unpublished results). Virus purified from plant sap also exhibited infectivity comparable to control preparations. The u.v. absorbance profiles of the three preparations with A280/A260 ratios of about 0·82 were essentially identical (Fig. 1), suggesting that little denaturation of viral protein occurred during chromatographic purification. The electrophoretic mobilities of coat protein from the three preparations were
also compared (Fig. 2). The coat proteins from all three virus preparations resolved into the characteristic fast- and slow-migrating bands typical of SMV (Hill & Benner, 1980). The relative electrophoretic mobilities of the bands were identical between treatments, and the molecular weights of the slow- and fast-migrating bands were estimated to be 30700 (± 100) and 29244 (± 100), respectively. These values are consistent with previous reports of the relative molecular weights of SMV coat protein bands in 12% polyacrylamide gels (Hill & Benner, 1980). Furthermore, SDS–polyacrylamide gels of SMV affinity-purified from plant sap contained no detectable bands other than those from SMV coat protein.

The sedimentation coefficients of affinity-purified and control viruses were essentially identical (approx. 144S) and are similar to values reported for potato virus Y (Delgado-Sanchez & Grogan, 1966) and tobacco etch virus (Purcifull, 1966), two other members of the potyvirus group. The antigenicities of the SMV preparations were found to be identical when tested in a monoclonal antibody-based ELISA. They reacted equally in the assay when tested at concentrations of 10 to 10000 ng virus/ml (Fig. 3).
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Fig. 3. Comparison of antigenicity of untreated (□), 'mock-purified' (○) and affinity-purified (●) SMV by using a monoclonal antibody-based ELISA. Capture S2 MAb was used at 275 ng/well, biotinylated S1 MAb at 60 ng/well and alkaline phosphatase-conjugated avidin was used at 12.5 ng/well. Values are averages of three determinations.

The morphology of SMV particles in the electron microscope in negatively stained preparations was unaffected by the affinity purification. Comparisons of affinity-purified SMV obtained from infected soybean leaves with that obtained from dilution of virus in uninfected plant extracts revealed no differences. The yield of virus purified from infected leaves was 3.6 mg per kg which is comparable to that of standard purification procedures.

DISCUSSION

Initial attempts to purify SMV by affinity chromatography were made by binding unprocessed ascitic fluid to an Affi-Gel 10 matrix and incubating the matrix with virus-containing sap for 30 min at room temperature. Small quantities of virus with typical potyvirus morphology were purified, but insufficient virus was obtained for detailed characterization. However, more virus was obtained when purified S2 IgG was immobilized onto Affi-Gel 10 beads, and the virus was allowed to react overnight with the immunoadsorbent. Under these conditions, approximately 0.4 mg of purified SMV was obtained from each addition of extract from infected plants to the column. By concentrating the virus from multiple cycles, it was possible to examine and compare several important characteristics of affinity-purified virus with virus purified by using standard procedures.

Previous work in our laboratory has shown that, although SMV is not stable at pH 3.0 in solutions containing 0.85% NaCl (McLaughlin et al., 1980) and dissociates at pH 3.0 in several other salt solutions (J. H. Hill, unpublished results), it is stable in distilled water adjusted to pH 3.0 with 0.1 M-HCl. This property has been used in the isolation of specific antibody to SMV.


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