Some properties of Potato Virus M (PVM) in Crude Sap and in Pure Preparations

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Key words: Potato Virus M; crude sap; pure preparations.

ABSTRACT

A study was performed to determine some properties of the potato virus M (PVM) in crude sap and in pure preparations. In crude sap the virus was shown to have a dilution end point value of $10^{-4}$, longevity in vitro particles sedimented as one peak with a sedimentation coefficient value of 162 s. The buoyant density in cesium chloride was 1.304 gm/cm$^3$. Electrophoresis in gel poliacrylamide gels showed that the virus possessed only one protein subunit with a molecular weight of 39,300 daltons.

INTRODUCTION

A virus identified as potato virus M (PVM) was isolated in British Columbia, Canada, and studies on host range (Ahmad et al., 1978) and purification (Ahmad, 1984) have been carried out. Some properties of the virus in crude sap and in pure preparations, albeit using different isolates, have been reported by several workers notably Bagnall et al. (1956), Hiruki (1973) and more recently Tavantzis (1982) and Ahmad (1984) (Table 1). However, the virus remains as one of the least characterised carlaviruses. Hence, this study aims to report some properties of the virus in crude sap and in pure preparations.

MATERIALS AND METHODS

Viruses

The PVM isolate used in this study was similarly maintained and purified as previously reported (Ahmad et al., 1978; Ahmad, 1984). Purified U$_1$ strains of tobacco mosaic virus (TMV) for the centrifugation experiment was kindly provided by Dr. J.A. Dodds.*

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TABLE 1

Some properties of potato virus M (PVM) in crude sap and in pure preparation

<table>
<thead>
<tr>
<th>Properties</th>
<th>Reported values (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle length (nm)</td>
<td>645 (g), 650 (c), 651 (a), 660 (f)</td>
</tr>
<tr>
<td>Particle width (nm)</td>
<td>13 (a, g)</td>
</tr>
<tr>
<td>Longevity (in vitro) (days)</td>
<td>2 (b, c, d, e), 3 (j)</td>
</tr>
<tr>
<td>Thermal inactivation point (C)</td>
<td>60 - 65 (h), 65 - 70 (b, c, e, j), 68 - 71 (i)</td>
</tr>
<tr>
<td>Dilution end point</td>
<td>(10^{-3}) (e), (10^{-4}) (d, h, j)</td>
</tr>
<tr>
<td>(A_{\text{max}}) (nm)</td>
<td>260 (a, g)</td>
</tr>
<tr>
<td>(A_{\text{min}}) (nm)</td>
<td>245 (a, g)</td>
</tr>
<tr>
<td>(A_{\text{max}}/A_{\text{min}})</td>
<td>1.21 (a, g), 1.23 (a)</td>
</tr>
<tr>
<td>Sedimentation coefficient</td>
<td>162 S (j) (^3)</td>
</tr>
<tr>
<td>Buoyant density (g/cm(^3))</td>
<td>1.304 (j) (^3)</td>
</tr>
<tr>
<td>Protein subunit molecular weight (daltons)</td>
<td>39,300 (j) (^3)</td>
</tr>
<tr>
<td>Estimated RNA content in virion (%)</td>
<td>5 - 6 (g), 6 (j)</td>
</tr>
</tbody>
</table>

\(^1\)Selected references only.

\(^2\)Sources of data: (a) Ahmad, 1984; (b) Bagnall et al., 1956; (c) Bagnall and Larson, 1957; (d) Hiruki, 1973; (e) Hario et al., 1969; (f) Rozendaal and van Slogteren, 1957; (g) Tavantzis, 1982; (h) Tu and Hiruki, 1970; (i) Wetter and Brandes, 1956; (j) present study.

\(^3\)Not previously reported.

**Physical Properties in vitro**

The dilution end point (DEP), longevity \(in vitro\) (LIV), and thermal inactivation point (TIP) were the properties of virus in crude sap being determined. Crude sap used for determining these properties was extracted from infected potato \((Solanum tuberosum\) L. designated Banana selection) or tomato \((Lycopersicon esculentum\) Mill. cv. Rutgers) plants. Extraction was achieved by macerating leaves of either plant in a mortar and then filtering it through muslin cloth.

The determination of DEP was performed with crude sap which has been diluted in 0.005 M borate buffer, pH 7.8, to \(10^{-1}\), \(10^{-2}\), \(10^{-3}\), \(10^{-4}\), \(10^{-5}\), or \(10^{-6}\) of the original concentration. The determination of TIP was carried out as follows: Two milliliters of crude sap were placed in thin-walled serological test-tubes \((75 \times 10\) mm) which were then incubated for 10 minutes in a water bath set at 30 C, 40 C, 50 C, 60 C, 65 C, 70 C, 75 C or 80 C. After 10 minutes the sap was immediately cooled by plunging the test-tubes into a beaker of ice for 30 seconds. In the determination of LIV, crude sap was incubated at room temperature for 1, 2, 3, 4, or 5 days.

The infectivity of the virus following each treatment was assayed onto the primary leaves of 10-12-day-old Red Kidney bean plants \((Phaseolus vulgaris\) L. cv. Red Kidney) (Hiruki, 1970). One half-leaf was inoculated with untreated freshly extracted sap while one-half leaf of the opposite primary leaf was inoculated with buffer only. The variously treated sap was then inoculated randomly onto the remaining half-leaves. The formation of local lesions was observed 6 - 12 days after inoculation.
Properties in Purified Preparations

Centrifugation experiments for the determination of sedimentation coefficient and buoyant density of the virus was carried out in a Spinco Model E analytical ultracentrifuge. Purified virus at concentrations ranging from 0.25 to 0.8 mg per ml in 0.005 M borate buffer, pH 7.8, was centrifuged at 21,740 rpm and 20 C. The sedimentation coefficient was then estimated graphically (Markham, 1960) using pictures of Schlieren patterns taken at four-minute intervals.

The buoyant density was estimated by the equilibrium method in cesium chloride (Chervenka, 1973). Test samples containing 30 - 50 µg of PVM and 436.9 mg of cesium chloride per ml of 0.005 M borate buffer, pH 7.8, were centrifuged for 12 hours at 44,770 rpm and 25 C. The actual densities of the cesium chloride solutions were determined from their refractive indices which were read using a refractometer. In some rotor cells, 100 µg of tobacco mosaic virus (TMV) U strain were added to PVM samples for comparison.

RESULTS

Physical Properties in vitro

Local lesions were observed on Red Kidney bean half-leaves which had been inoculated with sap diluted up to 10^{-4}. Thus, the DEP value of PVM in potato or tomato sap was 10^{-4}. In the experiment to obtain the LIV value, virus in crude sap was infective after 1, 2, or 3 days of incubation at room temperature prior to inoculation onto Red Kidney bean half-leaves. Virus in sap incubated for 4 days prior to inoculation did not show any infectivity indicating that the LIV value was 3 days. In another experiment, virus in sap heated for 10 min at 65 C or lower remained infective but virus in sap heated at 70 C was not infective. Thus, TIP of PVM from tomato or potato sap was 65 - 70 C.

Properties in Purified Preparations

Studies with purified preparations showed that the virus sedimented as one peak during analytical ultracentrifugation. The individual values of sedimentation coefficient obtained from six determinations ranged from 159 S to 166 S with a mean value of 162 S. The Schlieren pattern obtained from equilibrium banding of virus in cesium chloride showed that PVM particles formed a band closer to the centre of the rotor cell than did TMV particles (Fig. 1). The range of values obtained from four individual estimations was 1.301 - 1.309 g/cm^3 with a mean value of 1.304 g/cm^3. Tobacco mosaic virus which was run concurrently with PVM had a buoyant density of 1.324 g/cm^3.

Polyacrylamide Gel Electrophoresis

Dissociated virus moved through the polyacrylamide gel as one band. This indicated that PVM contained only one protein subunit. The molecular weight of the protein subunit differed slightly when different gel concentrations were used. The values estimated were 38,900, 39,100, 39,00, 39,600, 39,700 and 39,800 daltons for 4, 5, 6, 7, 8 and 9% polyacrylamide gels, respectively. The mean value for PVM protein subunit molecular weight was 39,300 daltons (Fig. 2).
DISCUSSION

The present study showed that the physical properties in vitro of PVM, such as a DEP value of $10^{-4}$ and a TIP value of $65 - 70$ °C were similar to some previously reported values (Table 1). However, LIV value of 3 days found in this study is slightly higher than the value of 2 days reported by Bagnal et al. (1956) and Hiruki (1973). This difference is probably not important since slight variations in physical properties in vitro of plant viruses is a common phenomenon especially when experiments were performed using different isolates (Francki, 1980).

This is the first report on sedimentation properties of PVM. The values of 162 s and 1.304 g/cm$^3$ for the sedimentation coefficient and the buoyant density, respectively, reported in this paper were typical properties of viruses of the carlavirus group (Matthews, 1981). These values indicated that PVM has an RNA content of about 6% (Gibbs and Harrison, 1976; Sehgal et al., 1976). The closedness of buoyant density estimation was confirmed by the value of 1.324 g/cm$^3$ obtained for the buoyant densities of TMV U, strain 1 are 1.325 g/cm$^3$ (Siegal and Hudson, 1959) and 1.324 g/cm$^3$ (Sehgal et al., 1970).

Although the molecular weight of the PVM protein subunit appeared to increase with higher gel concentrations, the differences were not significant ($P > 0.05$). The average value of 39,000 daltons was higher when compared with those of other carlaviruses for which a range from 31,000 – 36,700 daltons has been recorded (Wetter and Milne, 1981). In our tests, the PVM protein subunit consistently moved slower than alcohol dehydrogenase which has a molecular weight of 37,000 daltons (Fig. 3). This confirms the conclusion that the molecular weight of PVM protein subunits of other carlaviruses.

REFERENCES


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