An Icosahedral RNA Virus of the Soybean Looper (*Pseudoplusia includens*)

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**SUMMARY**

An icosahedral RNA virus isolated from *Pseudoplusia includens* is described. Purified virus preparations contained particles 40 nm in diam. with a sedimentation coefficient of 190S and a density of 1.33 g/ml. The virus consisted of 13 to 14% nucleic acid with a base ratio of A: 25.8%, U: 20.9%, G: 30.8%, C: 22.5%. Polyacrylamide gel electrophoresis demonstrated that the mol. wt. of the single-stranded RNA is 1.9 x 10^6 and the mol. wt. of the major polypeptide is 55000. The virus is not serologically related to the *Trichoplusia ni* and *Antheraea eucalypti* RNA viruses.

An icosahedral virus was isolated from larvae in our laboratory culture of soybean looper, *Pseudoplusia includens*, collected in Rapides Parish, La., U.S.A. The infection does not cause pronounced symptoms and was discovered during attempts to recover an icosahedral virus from beetles (Kim & Scott, 1978). When 'control' soybean loopers were extracted with phosphate buffer and injected into additional loopers, mild pathogenic effects were observed. Extraction and subsequent purification resulted in isolation of a virus which has been designated as the *Pseudoplusia includens* icosahedral virus (PIIV) whose production, purification, biophysical and biochemical properties are described herein. Although several viruses with morphological features similar to PIIV have been reported from Lepidoptera, the only other virus reported from *P. includens* was a baculovirus (Livingston & Yearian, 1972).

For virus production, fourth stage larvae were infected by injecting 1 μl of a suspension containing 400 to 500 μg/ml PIIV into the haemocoel. After inoculation larvae were maintained at 25 °C. Larvae were harvested after 7 days and frozen. The virus was extracted by blending 30 to 40 g larvae in 200 ml carbon tetrachloride and 400 ml 0.01 M-phosphate buffer pH 7.2, containing 0.2% diethylthiocarbamate. After a low-speed centrifugation of the homogenate, the virus was precipitated by making the aqueous phase 8% polyethylene glycol and 0.3 M-NaCl, resuspended in phosphate buffer and subjected to two alternate high- (78000 g for 30 min) and low- (7700 g for 10 min) speed centrifugations. The final purification step consisted of density gradient centrifugation (0-2 to 0.7 M-sucrose in 0.01 M-phosphate buffer pH 7-2) at 25000 rev/min for 2 h in a SW27 swinging bucket rotor. The virus fraction was recovered and pelleted by high-speed centrifugation.

Purified virus preparations were negatively stained using 2% (w/v) sodium phosphotungstate pH 5-2, and examined on a JEM-100 CX electron microscope. Large numbers of virus particles with a diameter of 40 nm were observed. The hexagonal outline of the particles suggests an icosahedral symmetry (Fig. 1). No core at the centre of the virus was seen when it was stained lightly. Occasionally, however, a central core was observed in heavily stained areas of the grid.

Purified PIIV (A_{260} = 1.0) in 0.01 M-phosphate buffer pH 7-2 was centrifuged at 28000 rev/min in the An-D rotor of the Beckman Model E analytical ultracentrifuge using u.v. optics. One centrifugal component with a sedimentation coefficient of 190S was observed. Buoyant density was determined by mixing purified virus (A_{260} = 0.04) with CsCl in 0.01 M-phosphate buffer pH 7-2, and centrifuging for 24 h at 44000 rev/min in the An-D rotor using u.v. optics. Virus density was determined by the method of Chervenka (1969). Density determination runs of PIIV gave a sharp band in the region of 1.33 g/ml.

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Fig. 1. Electron micrograph of purified *Pseudoplusia includens* icosahedral virus (PIIV) particles stained with 2% sodium phosphotungstate pH 5.2. Bar marker represents 100 nm.

Table 1. *Comparison of some physicochemical properties of Pseudoplusia includens icosahedral virus (PIIV) and the Nudaurelia capensis ß-virus (NßV) group*

<table>
<thead>
<tr>
<th>Property</th>
<th>PIIV</th>
<th>NßV group*</th>
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<tbody>
<tr>
<td>Size (nm)</td>
<td>40</td>
<td>35–38</td>
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<tr>
<td>Sedimentation coefficient</td>
<td>190</td>
<td>194–210</td>
</tr>
<tr>
<td>Density in CsCl (g/ml)</td>
<td>1.33</td>
<td>1.27–1.31</td>
</tr>
<tr>
<td>Mol. wt. of polypeptide (×10^-3)</td>
<td>55</td>
<td>61–68</td>
</tr>
<tr>
<td>Mol. wt. of RNA (×10^-6)</td>
<td>1.9</td>
<td>1.8–1.9</td>
</tr>
<tr>
<td>A_{260/280}</td>
<td>1.42</td>
<td>1.32–1.45</td>
</tr>
<tr>
<td>Percentage RNA</td>
<td>13–14</td>
<td>10–15</td>
</tr>
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</table>


The $A_{260}/A_{280}$ ratio of PIIV was 1.42 in 0.01 m-sodium phosphate buffer pH 7-2. This result indicates a nucleic acid content of about 13%. Calculation from the density determination (Sehgal *et al.*, 1970) revealed a 14% nucleic acid content. Nucleic acid extracted from purified virus with phenol–SDS (Poulson, 1973) reacted with orcinol but not with diphenylamine, revealing that the viral nucleic acid is RNA. The nucleotides were separated by paper chromatography (Wyatt, 1955), eluted in 0.1 M-HCl overnight, and read in the u.v. spectrophotometer at the specific absorption maxima. The base ratio was A: 25.8%, U: 20.9%, G: 30.8%, C: 22.5%. The percentage difference of base ratios between A, U and G, C demonstrates that this is a single-stranded nucleic acid.

Electrophoretic analysis of the nucleic acid in 2.9% acrylamide gel (Lane, 1974) using brome mosaic virus (mol. wt. 1.09, 0.99, 0.75, 0.28, all × 10^6) (Lane & Kaesberg, 1971) and tobacco mosaic virus (mol. wt. 2.1 × 10^6) (Lane, 1974) RNAs as markers revealed a single major RNA species of 1.9 × 10^6 mol. wt. The electrophoretic analysis of viral protein in 9% acrylamide gel revealed one major polypeptide with a mol. wt. of 55000 (Fig. 2a).

Antiserum was produced in rabbits given 9 weekly subcutaneous injections (1.5 mg virus per injection) of purified PIIV plus Freund's incomplete adjuvant. One band was typically observed in gel diffusion tests utilizing PIIV and its antiserum. Reciprocal comparisons between PIIV
Fig. 2. (a) Polyacrylamide gel analysis of PIIV protein. Lane 1, phosphorylase (mol. wt. 94K); lane 2, catalase (mol. wt. 60K); lane 3, aldolase (mol. wt. 40K); lane 4, polyhedrin of gypsy moth NPV (mol. wt. 30-4K), lanes 5 and 6, PIIV; lane 7, Trichoplusia RNA virus (TRV) (mol. wt. 67K). The solubilized proteins were electrophoresed on a 9% polyacrylamide slab gel using the discontinuous buffer system of Laemmli (1970). (b) Reciprocal comparison of PIIV (P) and TRV (T) and their antisera, PA and TA respectively, in gel diffusion plates, using 0.6% agarose in 0.01 M-sodium phosphate buffer pH 7.2, containing 0.02% sodium azide.

and Trichoplusia RNA virus (TRV) and its antiserum (kindly supplied by T. J. Morris) demonstrated no serological relationship (Fig. 2b).

P. includens icosahedral virus has several properties similar to certain other viruses infecting insects (Table 1). Reinganum et al. (1978) described three of these viruses: Darna trima virus, Thosea asigna virus and Philosamia cynthia × ricini virus. Based on the physical and chemical similarities and serological reactions, these three viruses were grouped with two others: Nudaurelia capensis β virus (NβV) (Juckes, 1970) and Antheraea eucalypti virus (Grace & Mercer, 1965) to form the NβV group. Additional members of this group have recently been described (Greenwood & Moore, 1982).

Although there are similarities between PIIV and the NβV group, there are important differences. The mol. wt. of the polypeptide of PIIV (55 000) is somewhat lower than that of the NβV group (61000 to 68000) (Table 1). This virus is also serologically unrelated to members of the NβV group, A. eucalypti RNA virus (C. Reinganum, personal communication) and TRV.

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REFERENCES


Short communication


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