A New Double-stranded RNA Virus from *Gaeumannomyces graminis*

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*(Accepted 14 February 1984)*

**SUMMARY**

An isometric virus, obtained from an isolate of *Gaeumannomyces graminis,* had the following properties: diameter 29 nm; sedimentation coefficient 127S; buoyant density in CsCl 1.40 g/ml; one double-stranded RNA component of 1.80 kilobase pairs; one capsid polypeptide species of mol. wt. 66000; $A_{260}/A_{280}$ 1.7. The virus was unrelated serologically to three other viruses present in the same fungal isolate and to 12 previously described viruses belonging to *G. graminis* virus groups I, II and III. On the basis of its distinctive properties the virus is assigned to a new *G. graminis* virus group IV.

Isometric virus particles in the wheat take-all fungus, *Gaeumannomyces graminis* var. *tritici,* were first discovered by Lapierre *et al.* (1970). Subsequently, Rawlinson *et al.* (1973) and Rawlinson & Muthyalu (1975) found that virus particles were of common occurrence in field isolates of this fungus. They found three sizes of isometric particles with diameters of 27 nm, 35 nm and 40 nm. Frick & Lister (1978) found considerable serotype variability among virus particles with modal diameters of 35, 39 and 41 to 42 nm obtained from 19 isolates of *G. graminis.* Later, 13 viruses from eight isolates of *G. graminis* were classified, on the basis of their serological and physical properties, into three groups, two of 35 nm viruses and the third of 40 nm viruses (Buck *et al.*, 1981). However, up to now none of the 27 nm particles has been purified or characterized. In the present communication I report the isolation of a *G. graminis* virus of 29 nm diameter and that its properties are distinct from those of previously described viruses in groups I, II and III. A fourth *G. graminis* virus group is proposed to accommodate this new virus.

*G. graminis* isolate 45/101, a spontaneous mutant of isolate 45/10 (Romanos *et al.*, 1980) which had previously been shown to contain particles of 35 nm and 27 nm diameter (C. J. Rawlinson, personal communication), was grown in 60 litre fermenters and virus particles were isolated and partially purified by polyethylene glycol precipitation and differential centrifugation as described by Buck *et al.* (1981). Further purification and fractionation of the virus particles were effected by sucrose density gradient centrifugation as described by Buck & Girvan (1977). Five fractions, shown by electron microscopy to contain isometric virus particles, were designated 1 to 5 in order of increasing rate of sedimentation and dialysed against 0.03 M-sodium phosphate buffer pH 7.6 containing 0.15 M-KCl (PK buffer).

Virus RNA was prepared from each sucrose fraction and shown to be double-stranded (ds) as described by Buck & Ratti (1975). Analysis and molecular weight determinations of dsRNA components by polyacrylamide gel electrophoresis (PAGE) and of capsid polypeptides by SDS—PAGE were carried out as described by Buck & Ratti (1977) and Buck *et al.* (1981). Particles in fraction 3 contained seven dsRNA components of 2-33, 2-27, 2-08, 2-02, 1-80, 1-70 and 1-53 kilobase pairs (kbp) (Fig. 1a, lane 2) and four polypeptide species with mol. wt. 73000, 68000, 66000 and 55000 designated P$_{73}$, P$_{68}$, P$_{66}$ and P$_{55}$ respectively (Fig. 1b, lane 1). Fraction 1 contained only the two smallest dsRNAs and predominantly P$_{55}$, while fraction 5 contained predominantly the four largest RNAs and the two largest polypeptides. These results suggested
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that $P_{33}$, $P_{68}$, $P_{66}$ and $P_{35}$ may belong to four distinct viruses, designated A, B, C and D respectively, assuming one capsid polypeptide species per virus.

For isolation of virus C, sucrose fraction 3 was mixed with CsCl to give a density of about 1.37 g/ml and centrifuged at 35000 rev/min for 21 h at 4 °C in a Beckman SW50.1 rotor. Examination of the gradient by overhead illumination revealed four light-scattering bands (Fig. 1 c). Fractions (0.05 ml) were collected from the bottom of the gradient and the density of each was measured by refractometry (Ifft et al., 1961). Small amounts of each fraction were spotted onto an agarose plate containing 6 mg/l ethidium bromide and examined for fluorescence on a u.v. transilluminator. No fluorescence was obtained from fractions in the top part of the gradient, indicating that the uppermost band contained no nucleic acid. Strong fluorescence was obtained from fractions in the top part of the gradient, indicating that the uppermost band contained no nucleic acid. Strong fluorescence was obtained in fractions corresponding to the other three bands which were designated 1, 2 and 3 (Fig. 1 c). Peak fractions were dialysed against PK buffer and examined by PAGE, SDS-PAGE, electron microscopy and sedimentation analysis. For electron microscopy, samples were negatively stained in 1% potassium phosphotungstate, pH 7.0 and examined in a Siemens Elmiskop 1A electron microscope which had internal calibration with a 69.6 μm aperture. Band 3 contained isometric particles of diameter 29.3 ± 1.9 nm (standard error, 115 particles), which had a similar appearance in electron micrographs to previously reported particles of 27 nm diameter from *G. graminis* (Rawlinson et al., 1973). The particles had a sedimentation coefficient ($s_{20}$ in PK buffer) of 127S, a buoyant density in CsCl of 1.40 g/ml, $A_{260}/A_{280}$ ratio of 1.7, a single dsRNA component of 1.80 kbp (Fig. 1 a, lane 4) and a single polypeptide species of mol. wt. 66000 (Fig. 1 b, lane 2); they thus correspond to virus C which is therefore the virus with particles of 27 nm diameter previously detected in *G. graminis* isolate 45/10.

Bands 1 and 2 from the CsCl gradient both contained $P_{33}$ and $P_{68}$ and dsRNA components of 2.33, 2.08 and 2.02 kbp in similar proportions (Fig. 1 a, lane 3) and particles of about 35 nm diameter. Both bands evidently contained viruses A and B. The reason for formation of two bands is unknown. For isolation of virus A, bands 1 and 2 were combined and dialysed against 0.01 M-Tris–HCl pH 8.0. This caused virus B and a proportion of virus A to precipitate out of solution. Virus A in the supernatant gave a single band of buoyant density 1.37 g/ml when
Table 1. Properties of virus particles in *G. graminis* virus groups I to IV

<table>
<thead>
<tr>
<th>Group</th>
<th>Particle diam. (nm)</th>
<th>( s_v ) (Svedbergs)*</th>
<th>Size of dsRNA components (kbp)†</th>
<th>Mol. wt. of capsid polypeptides (( \times 10^{-3} ))</th>
<th>( A_{260}/A_{280} ) ‡</th>
<th>Buoyant density in CsCl (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>35</td>
<td>109-128</td>
<td>1-59-1-89</td>
<td>54-60</td>
<td>1-4-1-5</td>
<td>1-36-1-37</td>
</tr>
<tr>
<td>II</td>
<td>35</td>
<td>133-140</td>
<td>2-02-2-33</td>
<td>68-73</td>
<td>1-4-1-5</td>
<td>1-35-1-37</td>
</tr>
<tr>
<td>III</td>
<td>40</td>
<td>159-163</td>
<td>5-1-6-0</td>
<td>78-87</td>
<td>1-6</td>
<td>1-40-1-41</td>
</tr>
<tr>
<td>IV</td>
<td>29</td>
<td>127</td>
<td>1-8</td>
<td>66</td>
<td>1-7</td>
<td>1-40</td>
</tr>
</tbody>
</table>

* Measured in PK buffer.
† A value of 687 for the mol. wt. of a base pair was used to convert mol. wt. values (Buck *et al.*, 1981) to kbp.
‡ Measurements were made at low concentrations (\( A_{260} \) about 0-5) and were not corrected for light scattering.

Virus D was unstable in CsCl, but could be isolated from sucrose fraction 1 by isopycnic centrifugation in a CsCl density gradient (40000 rev/min, 15 °C, 24 h, Beckman SW50.1 rotor). It had particles of diameter 34-4 ± 2-1 nm (standard error, 76 particles), \( s_v \) (in PK buffer) of 140S, \( A_{260}/A_{280} \) of 1-5, two dsRNA components of 2-33 and 2-27 kbp and one polypeptide species, \( P_{68} \). Virus B was deduced by elimination to contain the two dsRNA components of 2-08 and 2-02 kbp and the polypeptide \( P_{68} \).

Relationships of viruses A, B, C and D with each other and with previously described *G. graminis* viruses were examined by serology. An antiserum to viruses A, B, C and D was prepared by immunizing rabbits with sucrose fraction 3 and gel diffusion analyses were carried out as described by Buck *et al.* (1981). When allowed to diffuse against this antiserum viruses A, A + B mixture, C and D gave single precipitin lines with antiserum titres of 1:2048, 1:2048, 1:512 and 1:1024 respectively. When pairs of viruses were placed in adjacent wells virus C gave crossing precipitin lines with virus A, virus D and A + B mixture. Virus A gave a cross-precipitin line with virus D. It was concluded that virus C is serologically unrelated to viruses A, B and D and that virus A is serologically unrelated to virus D. Virus C did not react with any of six antiseras containing antibodies to a total of 12 *G. graminis* viruses from groups I, II and III (Buck *et al.*, 1981; McFadden *et al.*, 1983). However, virus A reacted with antiserum to virus T1-A (group II) and virus D reacted with antiserum to virus 019/6-A (group I).

The properties of viruses A and B are similar to those of group II viruses, while virus D has the properties of a group I virus (Buck *et al.*, 1981). The properties of virus C, however, are significantly different from viruses in groups I, II and III. The particles are smaller and have a dsRNA in the size range for a group I virus but with a capsid polypeptide size closer to that of a group II virus. Furthermore, the \( A_{260}/A_{280} \) ratio and buoyant density in CsCl are significantly greater than those of virus A, suggesting a higher dsRNA : protein ratio. Since data on these properties were not available for groups I, II and III viruses described previously (Buck *et al.*, 1981) they were determined for the following viruses. \( A_{260}/A_{280} \) ratios were as follows. Group I: 019/6-A, 1-4; 01-1-4-A, 1-4; 38-4-A, 1-5; OgA-B, 1-4. Group II: T1-A, 1-4; OgA-A, 1-4; 3bla-(B1 + B2), 1-4. Group III: 3bla-A, 1-6. Buoyant densities in CsCl (g/ml) were as follows. Group I: 019/6-A, 1-36; 01-1-4-A, 1-36; 38-4-A, 1-37; OgA-B, 1-36. Group II: T1-A, 1-35; OgA-A, 1-36. Group III: 3bla-A, 1-40 and 1-41 for particles containing dsRNAAs of 5100 and 6000 bp respectively. It is clear that the \( A_{260}/A_{280} \) ratio and buoyant density in CsCl of virus C are significantly higher than those of groups I and II viruses, although similar to those of the one group III virus measured. Virus C, however, has a much smaller particle diameter and smaller dsRNA and capsid polypeptide species than the group III viruses. It is therefore proposed to assign virus C to a new group, designated IV. Properties of the viruses in the four groups are compared in Table 1.

It is noteworthy that virus C apparently has only one dsRNA component. Furthermore, the full coding capacity of this RNA would be needed to encode the capsid polypeptide of mol. wt. 66000. By analogy with other dsRNA viruses it would be expected that at least one other gene...
would be needed to encode the RNA-dependent RNA polymerase needed for dsRNA transcription and replication. It is therefore possible that (i) virus C is a satellite virus dependent on one of the other viruses in the same isolate for replication, (ii) virus C has two dsRNA components of the same size but different sequence or (iii) virus C has a single dsRNA component with two or more genes encoded in different reading frames. If virus C is typical of *G. graminis* viruses of about 27 nm diameter it is unlikely to be a satellite since many *G. graminis* isolates contain only 27 nm particles (Rawlinson *et al.*, 1973). However, further studies on the molecular biology of virus C and other 27 nm viruses will be required to resolve these possibilities.

I am grateful to Miss M. B. Fitzgerald for assistance, Mr R. D. Woods for electron microscopy and Dr C. J. Rawlinson for stimulating discussions.

**REFERENCES**


(Received 16 November 1983)